PATHOGENIC

MICRO-ORGANISMS

INCLUDING

BACTERIA AND PROTOZOA

A PRACTICAL MANUAL FOR STUDENTS, PHYSICIANS AND HEALTH OFFICERS

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PREFACE TO FOURTH EDITION.

THE small volume which made the first edition of this book was called Bacteriology in Medicine and Surgery. It was written to make available for others the practical knowledge which had been acquired in the work of the bacteriological laboratories of the City of New York and was intended more for medical practitioners than for medical students or laboratory workers. When the first editions had been exhausted the improvement in methods of cultivating and studying the protozoa had reached a point rendering it advantageous to include the animal as well as the vegetable germs. This was done, and the title of the third edition was altered to conform with the text which had been broadened to cover the whole field of pathogenic microörganisms.

The book in its later editions has come to be used in an ever-increasing degree by medical students, so that while its point of view has remained the same, namely, to dwell especially on the relations of microörganisms to disease in man, it has been thought wise to touch on other aspects; thus, in this fourth edition a chapter has been added upon the bacteria concerned in agriculture and in some of the important fermentations. The bringing out of this new edition has enabled Dr. Williams and myself to rewrite a number of portions of the book with which we were not satisfied. We have also rearranged this material and added a number of tables which we believe will be helpful to the student. The chapters on the colon-typhoid group of bacilli and on malaria are examples.

Such subjects as the relation of bovine tuberculosis to that in man, the value of antimeningococcic serum, the use of bacterial vaccines, the etiology of anterior poliomyelitis and trachoma, and the prevention and cure of trypanosomiasis have been rewritten in the light of the new information which has been acquired since the writing of the preceding edition.

The revision of the different portions of the book has been divided between Dr. Williams and myself much as in the last edition. Dr. Williams has revised the portion of the book devoted to protozoa, while I have revised that on the pathogenic bacteria. We are greatly indebted to our associates in the laboratory for aid in many different ways.

This new edition, like its immediate predecessors, is intended to answer the needs of the students and physicians, and to cover the whole subject of pathogenic microörganisms from their standpoint.

W. H. P.

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PART I.

PRINCIPLES OF BACTERIOLOGY.

CHAPTER I.

INTRODUCTORY—HISTORICAL SKETCH.

ALTHOUGH most of the more important discoveries in bacteriology which place it on the footing of a science are of comparatively recent date, the foundations of its study were laid over two centuries ago. From the earliest times the history of bacteriology has been intimately associated with that of medicine. Indeed, it is only through the investigations into the life history of the vegetable and animal unicellular microörganisms that our present knowledge of the etiology, course, and prevention of the infectious diseases has been acquired. The prominent position which the study of microörganisms already holds toward medicine is, moreover, daily increasing in importance. Original discoveries are constantly adding to our knowledge of germ diseases, and the outlook is favorable for eventually obtaining, through serums, through attenuated cultures, or through the toxic substances produced by microörganisms themselves, means for immunizing against, if not curing, many of the specific infections. Even at present, bacterial products and protective serums are used successfully as preventive or curative agents in several of the most prevalent infectious diseases. Our knowledge concerning other microörganisms has enabled us largely to limit their dissemination and so to prevent disease. An acquaintance, therefore, with the main facts concerning these microörganisms is most necessary to the education of the modern physician.

But before entering into a detailed consideration of the subject it may be interesting and instructive to review very briefly a few of the important steps which led to the development of the science, and upon which its foundation rests, in which we shall see that the results obtained were gained only through long and laborious research and

after many obstacles were met and overcome by accurate observation and experiment.

Probably the first authentic observations of living microscopic organisms of which there is any record are those of Kircher, in 1659. This original investigator demonstrated the presence in putrid meat, milk, vinegar, cheese, etc., of "minute living worms," but did not describe their form or character.

Not long after this, in 1675, *Leeuwenhoeck* observed in rain-water, putrid infusions, and in his own and other saliva and diarrhœal evacuations living, motile "animalculæ" of most minute dimensions, which he described and illustrated by drawings. Leeuwenhoeck practised the art of lens-grinding, in which he eventually became so proficient that he perfected a lens superior to any magnifying glass obtainable at that day, and with which he was enabled to see objects very much smaller than had ever been seen before. "With the greatest astonishment," he writes, "I observed distributed everywhere through the material which I was examining animalcules of the most microscopic size, which moved themselves about very energetically." The work of this observer is conspicuous for its purely objective character and absence of speculation; and his descriptions and illustrations are done with remarkable clearness and accuracy, considering the imperfect optical instruments at his command. It was not until many years later, however, that any attempt was made to define the characters of these minute organisms and to classify them systematically.

From the earliest investigations into the life history and properties of bacteria, microörganisms have been thought to play an important part in the causation of infectious diseases. Shortly after the first investigations into this subject the opinion was advanced that puerperal fever, measles, smallpox, typhus, pleurisy, epilepsy, gout, and many other diseases were due to contagion. In fact, so widespread became the belief in a causal relation of these minute organisms to disease that it soon amounted to a veritable craze, and all forms and kinds of diseases were said to be produced in this way, upon no other foundation than that these organisms had been found in the mouth and intestinal contents of men and animals, and in water.

Among those who were especially conspicuous at this time for their advanced views on the germ-theory of infectious diseases was Marcus Antonius *Plenciz*, a physician of Vienna. This acute observer, who published his views in 1762, maintained that not only were all infectious diseases caused by microörganisms, but that the infective material could be nothing else than a living organism. On these grounds he endeavored to explain the variations in the period of incubation of the different infectious diseases. He also insisted that there were special germs for each infectious disease by which the specific disease was produced. Plenciz believed, moreover, that these organisms were capable of multiplication in the body, and suggested the possibility of their being conveyed from place to place through the air.

These views, it is true, were largely speculative, and rested upon insufficient experiment, but they were so plausible, and the arguments put forward in their support were so logical and convincing, that they continued to gain ground, in spite of considerable opposition and ridicule, and in many instances the conclusions reached have since been proved to be correct. The mode of infection, its unlimited development among large numbers of individuals, and gradual spread over wide areas—the incubation, course of, and resulting immunity in recovery from infectious diseases—all pointed to a living organism as the probable cause.

Among other distinguished men of the day whose observations exerted a most powerful influence upon the doctrine of infection, may be mentioned *Henle.* His writings (*Pathological Investigations,* 1840, and *Text-book of Rational Pathology,* 1853), in which he described the relation of microörganisms to infectious diseases, and defined the character and action of bacteria upon certain phases and symptoms of these affections, are remarkable for their clearness and precision.

But, meanwhile, the question which most interested these investigators into the cause of infectious diseases was: Whence are these microörganisms derived which were supposed to produce them? Were they the result of spontaneous generation due to vegetative changes in the substances in which the organisms were found, or were they reproduced from similar preëxisting organisms—the so-called vitalistic theory? This question is intimately connected with the investigations into the origin and nature of fermentation and putrefaction.

Spallanzani in 1769 demonstrated that if putrescible infusions of organic matter were placed in hermetically sealed flasks and then boiled the liquids were sterilized; neither were living organisms found in the solutions, nor did they decompose; and the infusions remained unchanged for an indefinite period.

The objection was raised to these experiments that the high temperature to which the liquids had been subjected so altered them that spontaneous generation could no longer take place. Spallanzani met the objection by cracking one of the flasks and allowing air to enter, when living organisms and decomposition again appeared in the boiled infusions.

Another objection raised by the believers in spontaneous generation was that, in excluding the oxygen of the air by hermetically sealing the flasks, the essential condition for the development of fermentation, which required free admission of this gas, was interfered with. This objection was then met by *Schulze,* in 1836, by causing the air admitted to the boiled decomposable liquids to pass through strong sulphuric acid. Air thus robbed of its living organisms did not produce decomposition.

Schwann in 1839 obtained similar results in another way: he deprived of microörganisms the air admitted to his boiled liquids by

passing it through a tube which was heated to a temperature high enough to destroy them. To this investigator is also due the credit of having discovered the specific cause—the yeast plant, or *saccharomyces cerevisiæ*—of alcoholic fermentation, the process by which sugar is decomposed into alcohol and carbonic acid.

Again it was objected to these experiments that the heating of the air had perhaps brought about some chemical change which hindered the production of fermentation. *Schroeder* and *von Dusch* in 1854 then showed that by a simple process of filtration, which has since proved of inestimable value in bacteriological work, the air can be mechanically freed from germs. By placing in the mouth of the flask containing the boiled solutions a loose plug of cotton, through which the air could freely circulate, it was found that all suspended microörganisms could be excluded, and that air passed through such a filter, whether hot or cold, did not cause fermentation of boiled infusions.

Similar results were obtained by *Hoffmann* in 1860, and by *Chevreul* and *Pasteur* in 1861, without a cotton filter, by drawing out the neck of the flask to a fine tube and turning it downward, leaving the mouth open. In this case the force of gravity prevents the suspended bacteria from ascending, as there is no current of air to carry them upward through the tube into the flask containing the boiled infusion.

Tyndall later showed (1876), by his well-known investigations upon the floating matters of the air, that the presence of living organisms in decomposing fluids was always to be explained either by the preëxistence of similar living forms in the infusion or upon the walls of the vessel containing it, or by the infusion having been exposed to air which was contaminated with organisms.

These facts have since been practically confirmed on a large scale in the preservation of food by the process of sterilization. Indeed, there is scarcely any biologic problem which has been so satisfactorily solved or in which such uniform results have been obtained; but all through the experiments of the earlier investigators irregularities were constantly appearing. Although in the large majority of cases it was found possible to keep boiled organic liquids sterile in flasks to which the oxygen of the air had free access, the question of spontaneous generation still remained unsettled, inasmuch as occasionally, even under the most careful precautions, decomposition did occur in such boiled liquids.

This fact was explained by Pasteur in 1860 by experiments showing that the temperature of boiling water was not sufficient to destroy all living organisms, and that, especially in alkaline liquids, a higher temperature was required to insure sterilization. He showed, however, that at a temperature of 110° to 112° C., which he obtained by boiling under a pressure of one and one-half atmospheres, all living organisms were invariably killed.

Pasteur at a later date (1865) demonstrated the fact that the organ-

isms which resist boiling temperature are, in fact, reproductive bodies, which are now known as *spores*.

In 1876 the development of spores was carefully investigated and explained by Ferdinand *Cohn*. He, and a little later *Koch*, showed that certain rod-shaped organisms possess the power of passing into a resting or spore stage, and when in this stage they are much less susceptible to the injurious action of higher temperatures than in their normal vegetative condition.

Stimulated by the establishment of the fact, through Pasteur's investigations, that fermentation and putrefaction were due to the action of living organisms reproduced from similar preëxisting forms, and that each form of fermentation was due to a special microörganism, the study of the causal relation of microörganisms to disease was taken up with renewed vigor. Reference has already been made to the opinions and hypotheses of the earlier observers as to the microbic origin of infectious diseases. The first positive grounds, however, for this doctrine, founded upon actual experiment, were the investigations into the cause of certain infectious diseases in insects and plants. Thus, *Bassi* in 1837 demonstrated that a fatal infectious malady of the silkworm—*pèbrine*—was due to a parasitic microorganism. Pasteur later devoted several years' study to an exhaustive investigation into the same subject; and in like manner *Tulasse* and *Kühne* showed that certain specific affections in grains, in the potato, etc., were due to the invasion of parasites.

Very soon after this it was demonstrated that microörganisms were probably the cause of certain infectious diseases in man and the higher animals. *Davaine*, a famous French physician, has the honor of having first demonstrated the causal relation of a microörganism to a specific infectious disease in man and animals. The anthrax bacillus was discovered in the blood of animals dying from this disease by *Pollender* in 1849 and by Davaine in 1850; but it was not until 1863 that the last-named observer demonstrated by inoculation experiments that the bacillus was the cause of anthrax.

The next discoveries made were those relating to wounds and the infections to which they are liable. *Rindfleisch* in 1866 and *Waldeyer* and *von Recklinghausen* in 1871 were the first to draw attention to the minute organisms occurring in the pyæmic processes resulting from infected wounds, and occasionally following typhoid fever. Further investigations were made in erysipelatous inflammations secondary to injury by *Billroth*, *Fehleisen*, and others, who agreed that in these conditions microörganisms could almost always be detected in the lymph channels of the subcutaneous tissues.

The brilliant results obtained by *Lister* in 1863–1870, in the antiseptic treatment of wounds to prevent or inhibit the action of infective organisms, exerted a powerful influence on the doctrine of bacterial infection, causing it to be recognized far and wide and gradually lessening the number of its opponents. Lister's methods were suggested to him by Pasteur's investigations on putrefaction.

In 1877 *Weigert* and *Ehrlich* recommended the use of the aniline dyes as staining agents and thus made possible a more exact microscopic examination of microörganisms in cover-glass preparations.

In the vear 1880 Pasteur published his discovery of the bacillus of fowl cholera and his investigations upon the attenuation of the virus of anthrax and of fowl cholera, and upon protective inoculation against these diseases. *Laveran* in the same year announced the discovery of parasitic bodies in the blood of persons sick with malarial fever, and thus stimulated investigations upon the immensely important unicellular animal parasites.

In 1881 Koch made his fundamental researches upon pathogenic bacteria. He introduced solid culture media and the "plate method" for obtaining pure cultures, and showed how different organisms could be isolated, cultivated independently, and, by inoculation of pure cultures into susceptible animals, could be made, in many cases, to reproduce the specific disease of which they were the cause. To him more than to any other are due the methods which have enabled us to prove absolutely, in a broad sense, the permanence of bacterial varieties. It was in the course of this work that the Abbe system of substage condensing apparatus was first used in bacteriology.

In 1882 Pasteur published his first communication upon rabies. The method of treatment devised by him is still in general use. A little later came the investigations of *Loeffler* and *Roux* upon the diphtheria bacillus and its toxins, and that of *Kitasato* upon tetanus. These researches paved the way for Behring's work on diphtheria antitoxin, which in its turn stimulated investigation upon the whole subject of immunity. The number of investigators rapidly increased as the importance of the earlier fundamental discoveries became apparent. Their additions to the science of bacteriology are considered in the pages of this book.

CHAPTER II.

GENERAL CHARACTERISTICS OF BACTERIA—CLASSIFICATION.

AMONG the microörganisms which have in common the ability to produce disease in animals and plants, the most important are the *Bacteria*. These minute organisms are usually classed as plants, but their structure is so simple and their biologic characteristics are so varied that their relationship to the vegetable kingdom is not clear-cut. In their possession of more or less rigid bodies, in the tendency of many to grow in filaments, and in the ability of some to use simple elements as food, they resemble plants; while in the motility of many, the non-possession by all of chlorophyll, and in the necessity of many for complex food, they resemble animals.

There is a similar difficulty in definitely classifying the other groups of closely related microörganisms, namely, the protozoa, the yeasts, and the moulds, and it has been suggested that under the name *Protista* a third kingdom be formed consisting of all of these lowest microörganisms.[1]

Definition of Bacteria.—*Bacteria* may be defined as extremely minute simple unicellular microörganisms, which reproduce themselves with exceeding rapidity, usually by transverse division, and grow without the aid of chlorophyll. They have no morphologic nucleus, but contain nuclear material which is generally diffused throughout the cell body in the form of larger or smaller granules.

Natural Habitat.—There are such wonderful differences in the conditions of life and nutrition which suit the different varieties, that bacteria are found all over the known world. Wherever there is sufficient moisture, one form or another will find other conditions sufficient for multiplication. Thus, we meet with bacterial life between 0° and 75° C. Some live only in the tissues of men, others in lower animals, a larger number may grow in both man and lower animals, others still grow only in plants, but by far the greater number live in dead organic matter. For some, free oxygen is necessary to life, for others, it is a poison.

Morphologic Characteristics of Bacteria.—The fact that each bacterial variety possible of cultivation may grow in distinctive ways upon so-called artificial culture media has been an immense aid in the differentiation of these microörganisms; for the individual cell of most varieties is so minute that even the highest magnification we have may show little if any morphologic differ-

[1] A discussion of the relationship between plants and animals is given in Ray Lankester's "Zoology," Vol. I, 1st Fascicle. Introduction, 1907. London. For the relationship to Protozoa see section III.

ence between organisms which produce distinctly different diseases, or between a pathogenic and a non-pathogenic form. There are, however, certain morphologic and biologic characteristics of the single cell which are pronounced, and we therefore study these before going on to the study of cultures, that is, of bacteria in masses.

The determination of morphologic characters for the description of bacteria should always be made from fully developed cultures; those which are too young may present immature forms, due to rapid multiplication, while in old cultures altered or degenerated forms may be observed.

When grown upon different media, variations, especially in size, may generally be observed. Such differences should always be described, together with a note of the media upon which the organism was developed and a statement as to whether each variation is a marked feature of the species under consideration.

The conditions of temperature and of nutrition which favor growth are quite various for different species, so that no fixed temperature, medium, or age of growth can be regarded as applicable to all species. Morphologic descriptions should always be accompanied by a definite statement of the age of the growth, the medium from which it was obtained, and the temperature at which it was developed.

The form and dimensions of bacterial cells at their stage of complete development must be distinguished from those which they possess just after or just before they have divided. As a spherical cell develops preparatory to its division into two cells it becomes elongated and appears as a short oval rod; at the moment of its division, on the contrary, the transverse diameter of each of its two halves is greater than their long diameter. A short rod becomes in the same way, at the moment of its division, two cells, the long diameter of each of which may be even a trifle less than its short diameter, and thus they appear on superficial examination as spheres.

Size.—The dimensions of the adult individual vary greatly in the different species as well as in members of the same species. The largest bacillus recorded is 50μ to 60μ[1] long and 4μ to 5μ wide (*B. Bütschlii*, see Fig. 15). One of the smallest forms known (*B. influenzæ*) is 0.5μ x 0.2μ. The average size of the known pathogenic rod-shaped bacteria is 2μ x 0.5μ, while that of the pathogenic cocci is about 0.8μ in diameter.

Some pathogenic organisms (supposed to be bacteria) are so small as to be invisible with any magnification which we now possess. We know of their existence only by the fact that they may be cultivated on artificial media, producing appearances of mass growth and that such cultures when inoculated into susceptible animals cause the characteristic disease (foot-and-mouth disease in cattle). These tiny organisms will pass through the pores of the finest Berkefeld filter.

A special method for the examination of so-called ultramicroscopic organisms has recently been devised, known as the dark-field illumi-

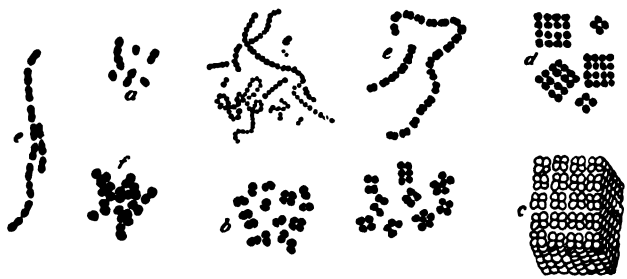
[1] A μ, or micromillimeter, is $\frac{1}{25000}$ of an inch.

nation (see p. 46). Micro-photography with ultraviolet light has also been employed, but so far very little has been learned by either of these means (see p. 47).

Shape.—The *basic forms* of the single bacterial cells are threefold—the sphere, the rod, and the segment of a spiral. Although under different conditions the type form of any one species may vary considerably, yet these three main divisions under similar conditions are constant; and, so far as we know, it is never possible by any means to bring about changes in the organisms that will result in the permanent conversion of the morphology of the members of one group into that of another—that is, micrococci always, under suitable conditions, produce micrococci, bacilli produce bacilli, and spirilla produce spirilla.

As bacteria multiply the cells produced from the parent cell have a greater or less tendency to remain attached. This is on account of the slimy envelope which is more or less developed in all bacteria. In some varieties this tendency is extremely slight, in others it is

FIG. 1



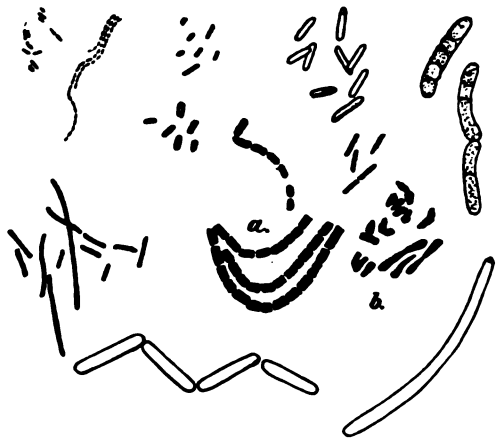
Varieties of spherical forms: *a*, tendency to lancet-shape; *b*, tendency to coffee-bean shape; *c*, in packets; *d*, in tetrads; *e*, in chains; *f*, in irregular masses. × 1000 diameters. (After Flügge.)

marked. This union may appear simply as an aggregation of separate bacteria or so close that the group appears as a single cell. According to the method of the cell division and the tenacity with which the cells hold together, there are different groupings of bacteria, which aid us in their differentiation and identification. Thus, in cocci we get the bacterial cell dividing into one, two, or three planes (Fig. 1), while in bacilli and spirilla the divison is generally in only one plane (Figs. 2 and 12).

1. SPHERICAL FORM, OR COCCUS (Fig. 1).—The size varies from about 0.3μ as minimum diameter to 3μ as maximum. The single elements are at the moment of their complete development, so far as we can determine, practically spherical; but when seen in the process of multiplication through division the form is seldom that of a true sphere. Here we have elongated or lancet-shaped forms, as frequently seen in the diplococcus of pneumonia, or the opposite, as in the diplococcus of gonorrhœa, where the cocci appear to be flattened against one another. Those cells which divide in one direction only and remain

attached are found in pairs (diplococci) or in shorter or longer chains (streptococci). Those which divide in two directions, one at right angles to the other, form bunches of four (tetrads). Those which divide in three directions and cling together form packets in cubes

FIG. 2



Various forms of bacilli: *a*, bacilli with sides parallel to their long axis and with ends perpendicular; *b*, bacilli with sides swollen or narrowed, causing irregular forms. × 1000 diameters. (After Flügge.)

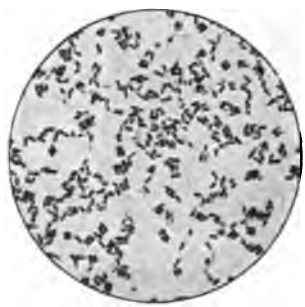
(sarcinæ). Those which divide in any axis form irregularly shaped, grape-like bunches (staphylococci).

2. ROD FORM, OR BACILLUS (Figs. 2, 3 and 4).—The type of this group is the cylinder. The length of the fully developed cell is always greater than its breadth. The size of the cells of different varieties varies

FIG. 3

Long slender bacilli.
× 1000 diam.

FIG. 4



Small bacilli, mostly in pairs.
× 1000 diam.

enormously: from a length of 30μ and a breadth of 4μ to a length of 0.2μ and a breadth of 0.1μ. The largest bacilli met with in disease do not, however, usually develop over 3μ x 1μ. Bacilli are roughly classed, according to their form, as slender when the ratio of the long

to the transverse diameter is from 1:4 to 1:10, and as thick when the proportions of the long to the short diameter is approximately 1:2.

The characteristic form of the bacillus has a straight axis, with uniform thickness throughout, and flat ends (Fig. 2 *a* and Fig. 5); but there are many exceptions to this typical form. Thus frequently the motile bacteria have rounded ends (Fig. 2); many of the more slender forms have the long axis, slightly bent; some few species, as for example the diphtheria bacilli (Fig. 2 *b* and Fig. 13), invariably produce many cells whose thickness is very unequal at different portions. Spore formation also causes an irregularity of the cell outline (Figs. 17 and 18).

FIG. 5

Large bacilli in chains. × 1000 diameters.

The bacilli except when they develop from spores or granules divide only in the plane perpendicular to their long axis. A classification, therefore, of bacilli according to their manner of grouping is much simpler than in the case of the cocci. We may thus have bacilli as isolated cells, as pairs (diplobacilli), or as longer or shorter chains (streptobacilli).

3. SPIRAL FORM, OR SPIRILLUM. —The members of the third morphologic group are spiral in shape, or only segments of a spiral. Here, too, we have large and small, slender and thick spirals. The twisting of the long axis, which here lies in two planes, is the chief characteristic of this group of bacteria. Under normal conditions the twisting is uniform throughout the entire length of the cell. The

FIG. 6

Medium-sized spirilla.

FIG. 7

Very large spirilla.

spirilla, like the bacilli, divide only in one direction. A single cell, a pair, or the union of two or more elements may thus present the appearance of a short segment of a spiral or a comma-shaped form, an S-shaped form, or a complete spiral or corkscrew-like form (Figs. 6 and 7).

The Higher Forms of Bacteria (see end of Section II).—A group of organisms intermediate between bacteria and the moulds have been

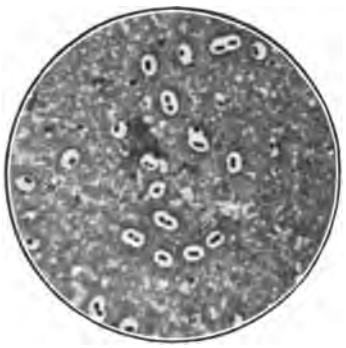
called higher bacteria. They show increased complexity of structure and function (1) in forming irregularly segmented filaments composed of elements similar to those found in the lower forms and showing either true or false branching, (2) in developing certain portions of their substance into reproductive bodies from which the new individuals grow.

The filaments seen sometimes among the lower forms have independent segments, which may easily separate and grow as tiny uncellular forms, while in the higher forms, the filaments in their growth show a certain interdependence of their parts. For example, growth often occurs from only one end of the filament while the other becomes attached to some fixed object.

The higher bacteria, therefore, show a close relationship to the fungi which have a still more complicated development. On the other hand, in their formation of gonidia, or swarm spores, during reproduction, they often present points of resemblance to the flagellata (see Protozoa).

Structure of Bacterial Cells.—When examined living in a hanging drop (see p. 41) under the microscope bacteria appear usually as colorless refractive bodies with or without spores or other more highly refractive areas. It is only by the use of stains that we are able to see more of their structure.

FIG. 8



Pneumococci with unstained capsules. From pneumonia sputum, stained with carbol-fuchsin and differentiated with weak acid alcohol. Magnification 1000. (Karg and Schmorl.)

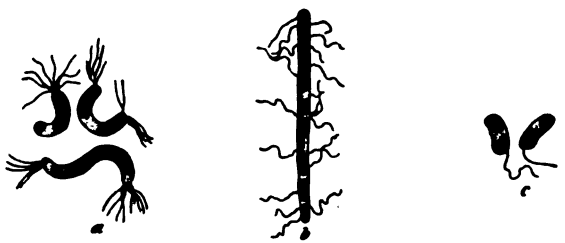
Capsule.—Special staining methods (see p. 33) show that many bacteria (some investigators say all) under certain conditions, possess a *capsule* (Fig. 8 and Fig. 18, p. 34), a gelatinous envelope which is supposed to be formed from the outer layer of the cell membrane. Some bacteria easily develop a much thicker capsule than others. Such forms are known as capsule bacteria. These generally produce a slimy growth on cultivation (*e. g., B. mucosus*).

Capsules develop best in animal tissues. In cultures, with a few exceptions, they require for their development special albuminous culture media, such as milk, blood serum, bronchial mucus, etc. In ordinary nutrient media or on potatoes the capsule may be visible in the first culture generations when grown from the body, but usually it shows very indistinctly if at all. The capsule is distinguished by a diminished power of staining with ordinary aniline dyes, therefore, unless special staining methods are used, the bacteria may appear to be lying in a clear unstained area. With certain dyes the inner portion of the capsule stains, giving the bacteria an apparent greater diameter. The demonstration of the capsule is often of help in differentiating between different but

closely related bacteria; *e. g.*, some forms of streptococcus and pneumococcus.

Cell Membrane.—That all bacteria possess a cell membrane is shown (1) by special staining methods (*e. g.*, flagella stains, see p. 35) and (2) by plasmolysis, demonstrated by placing the bacteria in a 1 per cent. solution of sodium chloride when the central portion (entoplasm?) contracts and separates in places from the membrane (Fig. 9). In some bacteria the membrane is slightly developed, while in others (*e. g.*, *B. tuberculosis*) it is well developed. It is different in composition from the membrane of higher plants in not possessing cellulose. In some forms, however, a similar carbohydrate, hemicellulose, has been demonstrated. In certain forms a substance related to chitin, found in the cyst walls of protozoa (Sec. III), has been found. Some observers consider the cell membrane merely a concentrated part of the cytoplasm, similar to the ectoplasm of higher cells. That it is closely related to the living part of the cell is shown by the connection of the organs of locomotion (flagella) with it.

FIG. 9



Plasmolysis: *a*, spirillum undula; *b*, bacillus solmsii; *c*, vibrio choleræ. The flagella are well shown. (After A. Fischer.)

The Cell Substance.—The nature and the structure of the cell substance contained within the membrane (body of bacteria proper, entoplasm) are still under discussion. The chief questions still unsolved relating to it may be summarized as follows: Is the bacterial cell similar to the higher cells in containing a definite nucleus surrounded by cytoplasm, or, if it is a simpler structure, does it behave more like a nucleus or more like cytoplasm?

In attempting a solution the following views have been expressed, chiefly after study of some of the larger bacteria:

1. Bacteria have a definite morphologic, more or less centrally situated nucleus (Feinberg, Nakanischi, Schottelieus, Swellengrebel, and others).
2. Bacteria have no nucleus or differentiated nuclear material (Fischer, Migula, Massart, and others).
3. The whole organism, except the membrane which is a delicate layer of cytoplasm, is a nucleus (Bütschli, Löwit, Boni, and others).
4. The nuclear material is in the form of distributed chromatin granules throughout the cytoplasm (Hertwig, Schaudinn, Guilliermond, Zettnow, and others).
5. A variety of the fourth view is that bacteria possess both chief elements of a cell, namely, cytoplasm and karyoplasm, but that these are so finely

mixed that they cannot be morphologically differentiated (Weigert, Mitrophanow, Gotschlich).

6. The latest view advanced, which is a variation of the views 3, 4, and 5, is that the bacterial cell is a relatively simple body—a cytode in Haeckel's sense, or the plasson of Van Beneden—which possesses both chromatin and plastin, the relative amounts of these chief substances of a cell corresponding more to the amounts found in the nuclei of higher cells than in their cytoplasm (Růžička, Ambrož).

These last authors call attention to the fact that both nucleus and cytoplasm in the higher cells are composed of a mixture of chromatin and plastin and that the chief difference between the two mixtures is one of amount and not of kind.

From our own studies of the structure of bacteria which have corrobated the views expressed in Nos. 4 and 6 of the above summary, we are certain that bacteria possess both chief elements of a cell, namely, chromatin and plastin, and that according to the stage of growth and division (varying with species) the chromatin may be in the form of morphologic granules, or may be so finely divided and mixed with the plastin as to be indistinguishable from it. At least some of the so-called *metachromatic granules* (Figs. 12 and 14) of many bacteria are undoubtedly nuclear in character. These granules appear in unstained bacteria as light-refracting, in stained preparations as deeply stained areas. They have a great affinity for dyes, and so stain readily and give up the stain with some difficulty. With complex stains they show a greater affinity than the rest of the bacillus for certain constituents of the stain—*e. g.*, with polychromic methylene blue they take up more of the azur, thus appearing red and indicating at the same time their nuclear nature. In certain bacteria, such as the diphtheria bacilli, they are especially well marked in young, vigorous cultures. Here they have diagnostic value.

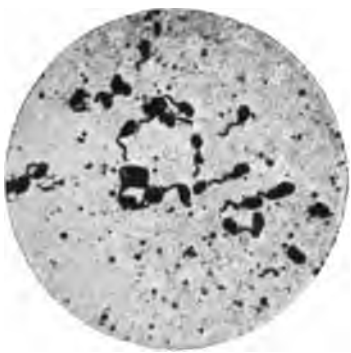
Besides the metachromatic granules there are certain other granules which take up stains readily and others still which absorb stains with difficulty; some of these granules are of the nature of starch and some of fat or other food products. Certain saprophytic forms have sulphur, others iron granules.

Organs of Motility—The outer surface of spherical bacteria, is almost always smooth and devoid of appendages; but that of the rods and spirals is frequently provided with fine, hair-like appendages, or *flagella*, which are organs of motility (Figs. 10 and 11). These flagella, either singly or in tufts, are sometimes distributed over the entire body of the cell, or they may only appear at one or both ends of the rod. The polar flagella appear on the bacteria shortly before division. The flagella are believed to be formed from the outer cell layer (ectoplasm) or possibly from the capsule, though they have been described by certain authors as arising in endoplasmic granules. They probably have the property of protrusion and retraction. So far as we know, the flagella are the only means of locomotion possessed by the bacteria. They are not readily stained, special staining agents being required for this purpose (see p. 35). The envelope of the bacteria, which usually

remains unstained with the ordinary dyes, then becomes colored and more distinctly visible than is commonly the case. Occasionally, however, some portion of the envelope remains unstained, when the flagella present the appearance of being detached from the body of the bacteria by a narrow zone. In stained cultures of richly flagellated bacteria peculiar pleated masses sometimes are observed, consisting of flagella which have been detached and then matted together. Bacteria may lose their power of producing flagella for a series of generations. Whether this power be permanently lost or not we do not know.

Bacteria are named according to the number and position of the flagella they possess as follows: *Monotricha* (a single flagellum at one pole; *e. g.*, cholera spirillum); *Amphitricha* (a flagellum at each pole; *e. g.*, many spirilla); lophotricha (a tuft of flagella at one pole;[1] *e. g.*, *Spirillum undulans*); peritricha (flagella projecting from all parts of surface; *e. g.*, *B. alvei*, *B. typhosus*, and others).

FIG. 10



Bacilli showing one polar flagellum.

FIG. 11

Bacilli showing multiple flagella.

So far, in only a few bacteria (the largest spirilla) have flagella been demonstrated during life, and then only under special conditions (see K. Reichert for bibliography). We have, however, an organism belonging to the *B. alvei* group, which shows its flagella very distinctly during life when a small portion of the viscid growth in a liquefying Löffler's blood-serum tube is transferred to a hanging mass of agar (p. 42) and examined under high magnification. The flagella on this organism may also be seen with dark-field illumination. In a recent article Reichert claims that all motile bacteria show their flagella by this method.

Physiologic Characteristics of Bacteria.—With the study of the organs of locomotion we pass naturally to the consideration of the essential physiologic activities of bacteria, namely, motility (irritability), growth, reproduction, and spore formation.

[1] Some investigators consider that every flagellum is essentially a tuft, composed of many small fibrils.

Motility.—Many bacteria when examined under the microscope are seen to exhibit active movements in fluids. The movements are of a varying character, being described as rotary, undulatory, sinuous, etc. At one time they may be slow and sluggish, at another so rapid that any detailed observation is impossible. Some bacteria are very active in their movements, different individuals progressing rapidly in different directions, while with many it is difficult to say positively whether there is any actual motility or whether the organism shows only molecular movements—so-called "Brownian" movements or *pedesis*—a dancing, trembling motion possessed by all finely divided organic particles. In order to decide definitely with regard to the motility of any bacterial preparation, it is well to make two hanging drops. To one, five per cent. of formalin is added, which of course kills the organism. If, now, the live culture shows motility, which is not shown by the killed culture, one may be certain that one is dealing with a motile culture. Very young cultures, of but three to four hours' development, in neutral nutrient bouillon should be examined at a temperature suitable for their best growth. Not all species of bacteria which have flagella exhibit at all times spontaneous movements; such movements may be absent in certain culture media and at too low or too high temperatures, or with an insufficient or excessive supply of oxygen; hence one should examine cultures under various conditions before deciding as to the non-motility of any organism.

The highest speed of which an organism is capable has been approximately estimated with some forms, and the actual figures show an actual slow rate of movement, though, comparatively, when the size of the organism is considered, the movement is rapid. Thus, the cholera spirillum may travel for a short time at the rate of 18 centimeters per hour.

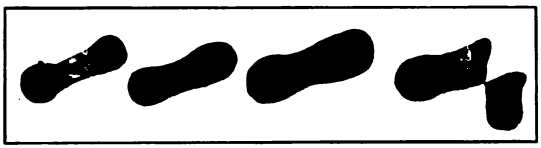
Movement is influenced by many factors, such as chemicals (the oxygen in the air especially), heat, light, and electricity. The tactile property which enables microörganisms to take cognizance of various forces is known as taxis; when forces attract, the phenomenon is known as positive taxis and when they repel it is called negative taxis. Chemotoxis, or the effect of chemicals, is taken up in detail on page 58.

Growth and Reproduction.—Under favorable conditions bacteria grow rapidly to a certain size, more or less constant for each species, and then divide by fission into approximated equal halves. The average time required for this cycle is twenty to thirty minutes. Probably in all species the nuclear material divides first. This is certainly the case in the group to which the *B. diphtheriæ* belongs where division of the nuclear granules may be observed in the living organism before the characteristic snapping of the cell body.

According to our observations on the living cell of members of this group, division takes place at a point occupied by a metachromatic granule (Fig. 12). Before division of the cell body the metachromatic granule, which appears to contain nuclear substance, elongates and shows a darker line at or near

its center. This seems to divide and form two lines, each of which has at a point near the surface a very tiny, refractive granule, staining deeply with chromatin stains. Between these two lines the cell body suddenly divides with a snap, like the opening of a jackknife, division beginning at the point between the two tiny granules, and the two new cells remain for a variable time attached at opposite points, thus giving the V-shaped forms. Kurth and Hill also called attention to division by snapping in members of the diptheria bacillus group, though neither recognized the relation between the position of the metachromatic granules and the point of division. The tiny granules are probably similar to the cell-partition granules described by various observers.

FIG. 12



1 2 3 4

Successive stages in division of B. diphtheriæ showing relation of line of division to metachromatic granule. Continuous observation of living bacillus drawn without camera lucida. (Williams.)

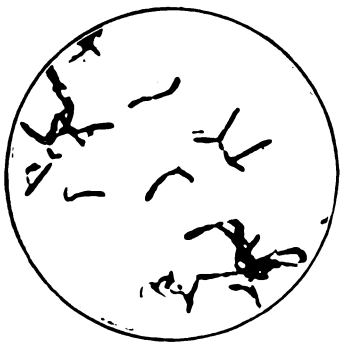
It is very seldom that the favorable conditions mentioned above for the production of equal and rapid division obtain for any time, since even in pure cultures bacteria in their growth soon produce an environment unfavorable for further multiplication. Several factors help to make this environment: First, the using up of suitable food and moisture; second, the disintegration of food substances into various injurious products, such as acids, alkalies, ferments; third, in mixed cultures the overgrowth of one or more varieties. As these unfavorable conditions are more or less constantly present, we seldom see such absolute symmetry in the growth and divison of bacteria as is usually described. In fact, except under ideally favorable conditions (*e. g.*, rapid successive transfers from young cultures on the most favorable food medium), we can never see absolutely equal fission among bacteria; and in some species, notably the diphtheria group, division is extremely irregular even in our usual twenty-four cultures on favorable media.

Involution and Degeneration Forms.—It follows, from the conditions considered above, that, as cultures grow older or when media unfavorable to equal division are used, the bacteria may show extremely irregular forms, absolutely different from the young forms, such as long threads or filaments with irregular thickenings, coccus forms from bacilli and spirilla which have divided without increasing in length, bacillar forms from cocci which have grown without dividing, and apparently branched forms from many varieties of bacilli and spirilla. These have been called *involution* or *degenerative forms.*

In our study of the so-called branched forms of the diphtheria bacillus we have observed the following interesting fact. Under certain conditions, marked apparent branching appears at a definite time in the age of the culture. The conditions are, slightly disturbed growth in pellicle on nutrient broth.

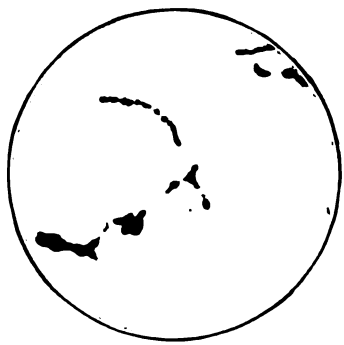
When such pellicles are examined every day they are found to contain, from the sixth to the twelfth day, varying chiefly with the amount of disturbance, many large intensely staining forms with one to several apparent branches and many large metachromatic granules (Figs. 13 and 14). The facts that these forms were the only ones to show active growth and divison when ex-

FIG. 13



B. Diphtheriæ "No. 8" from 9 days' broth pellicle, showing many "branched" forms. Stained with carbol-fuchsin. × 1500 diameters.

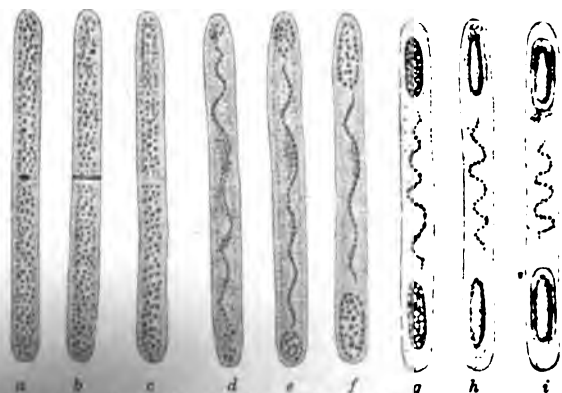
FIG. 14



B. Diphtheriæ "No. 8" from 10 days' broth pellicle, showing longitudinal fusion and position of metachromatic granules. Stained with Löffler's methylene blue. × 2000 diameters.

amined on a hanging mass of agar and that in such growth the metachromatic granules seem to fuse (Fig. 14) before fission led us to suppose that these forms represent a primitive sexual process, a sort of autogamy. Schaudinn (Fig. 15) has shown a primitive conjugation (autogamy) and a relationship between the chromatin granules, or nuclear substance, and the spores in certain bacteria.

FIG 15.



Bacillus Butschlii: *a* to *c*, incomplete division of the cell; *d* to *f*, gradual collection of chromatin granules at ends of cells; *g* to *i*, formation of end spores from these chromatin end masses. (After Schaudinn.)

Although ele[illegible] the greater diameter and complete division at righ[illegible] the rule for the majority of bacteria, there are c[illegible] instead of becoming separated from each other [illegible] produce an incomplete segmentation, the cells

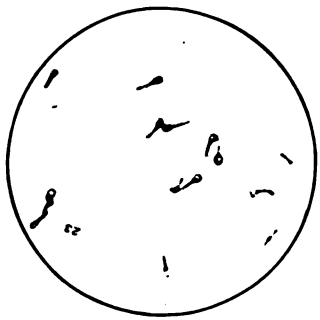
remaining together in masses, as the sarcinæ, for example, which divide more or less regularly in three directions. The indentations upon these masses or cubes, which indicate the point of incomplete fission, give to these bundles of cells the appearance commonly ascribed to them—that of a bale of rags. As already said, incomplete division in two opposite directions results in the formation of a group of forms as tetrads. Division irregularly in different directions without subsequent separation of the daughter cells results in the production of clusters; similar clusters are also formed when transversely-dividing organisms remain partly attached and are pushed slightly from their position. The rod-shaped bacteria never divide longitudinally.

Spore formation must be distinguished from vegetative reproduction. This is the process by which the organisms are enabled to enter a stage in which they resist deleterious influences to a much higher degree

FIG. 16

Unstained spores in slightly distended bacilli. (The spores are the light oval spaces in the heavily stained bacilli.)

FIG. 17



Unstained spores in distended ends of bacilli.

than is possible for them to do in the growing or vegetative condition. It is true that in all non-spore bearing cultures a certain proportion of the bacteria are more resistant than the average. No marked difference in protoplasm, however, has been noted in them other than the ability to stain more intensely and sometimes to show strong metachromatic areas. The difference between these and the less resistant forms is not great. Some have believed that this resistance is due to certain bodies called *arthrospores*, which are abnormally large cells with, usually, a thickened cell wall and increased staining properties, formed as a rule in old cultures. Fullerton and others have described similar forms in some of the higher bacteria and consider them spores. See nocardia (streptothrix.) The true spores of the lower bacteria are definite bodies. These are strongly refractile and glistening in appearance, oval or round in shape, and composed of concentrated protoplasm developed within the cell and surrounded by a very dense envelope (Figs. 16 and 17). They are characterized by their power of resisting the injurious influences of heat, desiccation, and chemical disin-

fectants up to a certain limit. (See p. 103 for details.) Spores also stain with great difficulty.

The production of endospores in the different species of bacteria, though not identical in every instance, is very similar. The conditions under which they are produced in nature are supposed to be similar to those observed in artificial cultures, but they may not always be similar, hence we must not consider a bacterium a non-spore bearer because it has not been seen to form spores in the laboratory. Usually the formation of spores in any species is best observed in a streak culture on nutrient agar or potato, which should be kept at the temperature nearest the optimum for the growth of the organism to be examined. At the end of twelve, eighteen, twenty-four, thirty, thirty-six hours, etc., specimens of the culture are observed, first unstained in a hanging drop or on an agar mass, and then, if round or oval, highly refractile bodies are seen, stained for spores. Each bacillus, as a rule, produces but one spore, and more than two have never been observed.

Motile bacteria usually come to a state of rest or immobility previous to spore formation. Several species first become elongated. The anthrax bacillus does this, and a description of the method of its production of spores may serve as an illustration of the process in other bacteria. In the beginning, the protoplasm of the elongated filaments is homogeneous, but after a time it becomes turbid and finely granular. These fine granules are then replaced by a smaller number of coarser granules, the so-called sporogenous granules, supposed to be chiefly nuclear in nature, which by coalescence finally amalgamate into a spherical or oval refractive body. This is the spore. As soon as the process is completed there may appear between each two spores a delicate partition wall. For a time the spores are retained in a linear position by the cell membranes of the bacilli, but these are later dissolved or broken up and the spores are set free. Not all the cells that make the effort to form spores, as shown by the spherical bodies contained in them, bring these to maturity; indeed, many varieties, under certain cultural conditions, lose altogether their property of forming spores. The following are the most important spore types: (*a*) the spore lying in the interior of single, short, undistended cells; (*b*) the spores lying in the interior of a chain of undistended cells; (*c*) the spore lying at the extremity of a cell much enlarged at that end—the so-called "head spore" or plectridium, *e. g.*, the tetanus bacillus (Fig. 17); and (*d*) the spore lying in the interior of a cell very much distended in its central portion, giving it a spindle shape or clostridium, *e. g.*, *Bacillus butyricus.*

According to Schaudinn and others, in certain spore bearing bacteria the spore formation is part of a sexual-like process (see under Reproduction).

The *germination of spores* takes place as follows: By the absorption of water they become swollen and pale in color, losing their shining, refractive appearance. Later, a little protuberance is seen upon one side (equatorial germination) or at one extremity of the

spore (polar germination) and this rapidly grows out to form a rod which consists of soft-growing protoplasm enveloped in a membrane, which is formed of the endosporium or inner layer of the cellular envelope of the spore. The outer envelope, or exosporium, is cast off and may be seen in the vicinity of the newly formed rod.

The chief spore formers among the pathogenic bacteria are the anaërobes (tetanus, malignant, œdema, intestinal bacteria). Only one distinctly pathogenic aërobe produces spores—the anthrax bacillus.

Reproduction Among the Higher Bacteria.—These forms increase in length for a time and then, at the free ends, or at intervals along the filaments, they produce small rounded cells, called gonidia or spores from which new individuals are formed. The terminal spores may be flagellated after their separation from the parent filament.

The flagellated forms frequently resemble certain flagellata among the protozoa.

CHEMICAL COMPOSITION OF BACTERIA.

Qualitatively considered, the bodies of bacteria consist largely of water, salts (chiefly phosphorus, potassium, chlorine, calcium and sulphur), fats, and albuminous substances. There are also present, in smaller quantities, extractive substances soluble in alcohol and in ether. Special varieties contain unusual substances, as wax and hemicellulose in tubercle bacilli. Bacteria possess the capacity in a high degree of accommodating their chemical composition to the variety of soil in which they are growing. The same variety of bacteria thus varies greatly in the quantitative estimation of its chemical constituents. Each variety, furthermore, yields proteid substances peculiar to itself, as shown in the effects produced by animal inoculation. At present we know but little concerning the differentiation of these specific substances. This subject will be taken up in detail under bacterial toxins, etc. According to Cramer, many bacteria contain amyloid substances which give a blue reaction with iodine. True cellulose has not been found in bacteria, but large quantities of a gelatinous carbohydrate similar to hemicellulose have been obtained. Nuclein is found frequently. The nuclein bases—xanthin, guanin, and adenin—have been obtained in considerable amounts. There is a group of bacteria which contain large amounts of sulphur—viz., the *Beggiatoa*—and another group, the *Cladothrix*, is capable of separating ferric oxide from water containing iron.

Some light has been thrown upon the chemical composition of bacteria, quantitatively, by the studies of Cramer, though so far only a few species have been thoroughly investigated. The percentage of water contained in bacteria grown on solid culture media, as well as the amount of residue and ash, depends largely on the composition of the media. Thus, *Bacillus prodigiosus* when grown on potato contains 21.5 per cent. of dry residue and 2.7 per cent. of ash; when cultivated on turnips it contains 12.6 per cent. of dry

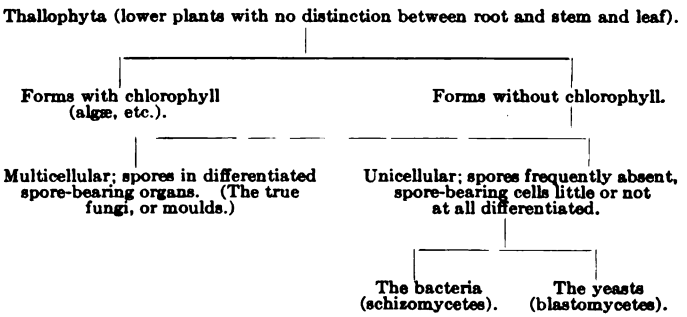
residue and 1.3 per cent. of ash. Besides the concentration of the culture, its temperature and age also influence the amount of residue and ash produced. The residue varies, moreover, qualitatively in the same species under the influence of the culture media employed. Thus, it appears that an additional quantity of peptone in the culture media tends to increase the percentage of nitrogenous matter in the bacillus, while the addition of glucose decreases it.

Microchemical Reactions.—To a certain degree the chemical composition of the *individual* bacterium may be studied both in the living and in the dead organism by the addition of the testing substances to a hanging drop or to a spread of such organism and the examination of it under the microscope.

Of special importance in this regard is the resistance which bacteria possess to diluted alkalies. Inasmuch as the majority of animal tissues are dissolved when treated with alkalies, this method has been adopted for rendering visible unstained bacteria in tissues. As a rule, bacteria are stained yellowish with iodine solution, a few only in consequence of their starchy constituents being stained blue. (See also Principles of Staining Bacteria, p. 30.)

CLASSIFICATION OF BACTERIA.

The position of the bacteria at the lower end of plant life and their relationship to the next higher plants may be seen in the following table:



Bacteria themselves have been classified in many different ways by different observers. As a rule, the genera are based upon morphologic characters and the species upon biochemic, physiologic, or pathogenic properties. While the form, size, and method of division are the most permanent characteristics of bacteria, and should be naturally utilized for classification, nevertheless, in this basis of division, because of the minute size of the organisms and of our consequent inability to detect important morphologic differences, there are decided difficulties. Then, too, though the form and size of the different varieties are fairly constant under the same conditions, under diverse conditions as we have already noticed, they may be quite different. Another serious

drawback for our purposes is that these morphologic characteristics give no indication whatever of the relations of the bacteria to disease and fermentation—the chief characteristics which give them their importance to human beings. The properties of bacteria which are fairly constant under uniform conditions and which have been more or less used in systems of classification are those of spore and capsule formation, motility (flagella formation), reaction to staining reagents, relation to temperature, to oxygen, and to other food material, and, finally, their relation to fermentation and disease.

But any one of these properties under certain conditions may so vary that, taking it as a basis for classification, an organism could be dropped from the group with which it had been classified and be placed in an entirely different group.

Thus, the power to produce spores or flagella may be held in abeyance for a time or, in the case of the former, be totally lost; the relations to oxygen may be gradually altered, so that an anaërobic species grows in the presence of oxygen; parasitic bacteria may be so cultivated as to become saprophytic varieties, and those which have no power to grow in the living body may acquire pathogenic properties.

The possibility of making any thoroughly satisfactory classification is rendered still more difficult by the fact that many necessarily imperfect attempts have already been made, so that there is a great deal of confusion, which is steadily increased as new varieties are found or old ones reinvestigated and classified differently in the different systems.

As one of the more successful attempts to classify bacteria, the system devised by Migula is here given, simply as an example. The morphology of bacteria is used as the basis of the division:

	FAMILIES.
I. Cells globose in a free state, not elongating in any direction before division into 1, 2, or 3 planes..	1. Coccaceæ.
II. Cells cylindrical, longer or shorter, and only dividing in one plane, and elongating to about twice the normal length before the division.	
a. Cells straight, rod-shaped, without sheath, non-motile, or motile by means of flagella.....	2. Bacteriaceæ.
b. Cells curved, without sheath.....	3. Spirillaceæ.
c. Cells enclosed in a sheath.....	4. Chlamydobacteriaceæ.

	GENERA.
1. *Coccaceæ*.	
Cells without organs of motion.	
a. Division in one plane.....	1. Streptococcus.
b. Division in two planes.....	2. Micrococcus.
c. Division in three planes.....	3. Sarcina.
Cells with organs of motion.	
a. Division in two planes.....	4. Planococcus.
b. Division in three planes.....	5. Planosarcina.
2. *Bacteriaceæ*.	
Cells without organs of motion.....	1. Bacterium.
Cells with organs of motion (flagella).	
a. Flagella distributed over the whole body.....	2. Bacillus.
b. Flagella polar.....	3. Pseudomonas.

3. *Spirillaceæ.*

Cells rigid, not snake-like or flexuous.	
a. Cells without organs of motion	1. Spirosoma.
b. Cells with organs of motion (flagella).	
1. Cells with 1, very rarely 2 to 3 polar flagella	2. Microspira.
2. Cells with polar flagella-tufts	3. Spirillum.
Cells flexuous	4. Spirochæta.

4. *Chlamydobacteriaceæ* (higher bacteria, also known as Trichomycetes).

Cell contents without granules of sulphur.	
a. Cell threads unbranched.	
I. Cell division always only in one plane	1. Streptothrix.
II. Cell divsion in three planes previous to the formation of gonidia.	
1. Cells surrounded by a very delicate, scarcely visible sheath (marine)	2. Phragmidiothrix.
2. Sheath clearly visible (in fresh water)	3. Crenothrix.
b. Cell threads branched	4. Cladothrix.
Cell contents containing sulphur granules	5. Thiothrix.

The above table makes changes in the designation of some of the most common bacteria, as in the restoration of the old title bacterium and the assigning it to all of the non-motile, rod-shaped organisms, thus altering the name of some of the most common pathogenic bacteria from bacillus to bacterium. Other changes are seen in the spirilla, and the classification of the higher bacteria is quite different from that now accepted (see end of Sec. II). Any such scheme is at times arbitrary in placing some varieties under one generic division and others closely allied in another. It has also the objection, already noted, that it is only one of several classifications already in use, and until an authoritative body agrees on some one, it seems unwise in such a volume as this to change the usually employed names for others which are, perhaps, intrinsically better. Another important reason for waiting is that with the increase of our knowledge we are constantly changing the position of different bacteria. Thus, such a well-known germ as the tubercle bacillus is now found to produce, under certain conditions, long, thread-like branching forms; so that it ceases to be under the classification of Migula, either a bacillus or bacterium. We shall, therefore, simply use in this book the older, less scientific nomenclature, of classing all rod forms as bacilli and all spiral forms as spirilla, and consider together, in so far as is practicable, certain groups of bacteria whose members are closely allied to each other in some one or more important directions.

It is well to call attention, however, to the fact that in naming bacterial species the binomial law of nomenclature has been frequently violated. Such names as *Bacillus coli communis* should not be accepted; the name *Bacillus coli* is sufficient as well as correct.

Permanence of Bacterial Species.—When we come to study special varieties or groups of bacteria, such as the bacilli which produce typhoid fever, diptheria, and tuberculosis, it is of great importance for us to determine, if possible, to what extent the peculiar characteristics which each of these groups of bacteria possess are permanent in the generations which develop from them.

We cannot believe that the multitude of bacterial varieties which now exist have always existed. The probability is very strong that with succeeding generations and changing conditions new bacterial varieties have developed with new characteristics.

From time to time the changing conditions under which life progresses probably expose certain animals to the invasion of varieties which never before have gained access to them. If the bacteria find some means of transmission to other animals equally susceptible, a parasitic species becomes established which at first, perhaps, finds conditions only occasionally favorable to it. Thus in some such way a multitude of bacterial groups have arisen, some of which accustom themselves to the conditions present in living plants, others to those in fishes, others to those in birds, and others still to those in man and the higher animals.

These are, however, theories. What has been actually observed in the few years during which bacteria have been studied? In this short time the pathogenic species as observed in disease have remained practically unaltered. The diptheria bacilli are the same to-day as when Loeffler discovered them in 1884, and the disease itself is evidently the same as history shows it to have been before the time of Christ. The same permanence of disease type is true for tuberculosis, smallpox, hydrophobia, leprosy, etc. Under practically unchanged conditions, therefore, such as exist in the bodies of men, bacteria which have once become established as parasites, continue to reproduce new generations which retain their peculiar (specific) characteristics. It is true that among the countless organisms developed some fail to hold the parasitic characteristics. These either continue as saprophytes or cease to exist. Whether new disease varieties are coming into existence from time to time is, of course, a possibility, but not a certainty. The one thing we can probably safely assert is that it is very unlikely that any saprophytic variety now existing can develop into the now recognized varieties of pathogenic bacteria. It is difficult to conceive that any such variety should develop parasitic tendencies under exactly the same circumstances as those varieties which now produce disease.

The fact that the chief pathogenic varieties of bacteria which excite disease in man seem to have retained for centuries their characteristics, in no way proves that when placed under different conditions they would remain stable. As already stated, certain characteristics of some bacteria can be radically altered by changed conditions, such as being grown outside the natural host, either in the test-tube or in an unaccustomed host. When these new surroundings are unfavorable, the organisms, while retaining their morphology, may lose their power of developing and producing specific poisons in the original host. Such *attenuation* may also occur in certain organisms when retained for a long time in an apparently immune host, as is seen in the streptococci and pneumococci of the throat or in the colon bacilli of the intestines.

The *recovery of poison production* is often brought about by developing

the microörganism for a considerable length of time under the conditions best suited for it. The *recovery of the ability to grow in the body* of any animal species is brought about by causing the germ to develop in a series of animals of the same species whose resistance has been overcome by reducing their vitality through poisons, heat, cold, etc., or by giving enormous doses of bacteria to produce the first infection. Another method is to accustom the microörganism to the animal's body by letting it remain surrounded by the animal fluids but protected from phagocytes in a pervious capsule in the peritoneal cavity or by growing it in unheated fresh serum or blood media.

The above examples of variations may be classed under those known as fluctuating variations. True mutations or discontinuous variations among bacteria have been very seldom observed.

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CHAPTER III.

MICROSCOPIC METHODS.

DRY AND MOIST PREPARATIONS, STAINS, AND MICROSCOPIC EXAMINATION OF BACTERIA.

THE direct microscopic examination of suspected substances for bacteria can be made either with or without staining. Unstained, the bacteria are examined living in a hanging drop or on transparent solid media, under daylight, or, better, artificial light, to note their number, their motility, their size, form, and spore formation, their general arrangement and their reactions to specific serums; but for more exact study of their structure they can be so much better observed when stained in a dried film preparation on a glass slide or a cover-glass that this step is always advisable.

Elimination of Foreign Bacteria from Preparations.—Since bacteria are present in the air, in dust, in tap water, on our bodies, clothes, and on all surrounding objects, it follows that when we begin to examine substances for bacteria the first requisite is, that the materials we use, such as staining fluids, cover-glasses, etc., should be practically free from bacteria, both living and dead, otherwise we may not be able to tell whether those we detect belong originally in the substances examined or only in the materials we have used in the investigation.

Film Preparation (spread, smear).—A cover-glass or slide preparation is made as follows: A very small amount of the blood, pus, discharges from mucous membranes, cultures from fluid media, or other material to be examined is removed, usually by means of a sterile swab or platinum loop, and smeared undiluted in an even, thin film over a perfectly clean,[1] thin cover-glass or slide. From cultures on solid media, however, on account of the abundance of bacteria in the material, a little

[1] To render new cover-slips clean and free from grease, the method recommended by Gage is useful: Place in following solution overnight.

Bichromate of potash ($K_2Cr_2O_7$)	200 grms.
Water, tap or distilled	800 c.c.
Sulphuric acid	1200 c.c.

The bichromate is dissolved in the water by heating in agate kettle; the sulphurous acid is added very slowly and carefully on account of great heat developed. After cooling, it is kept in glass vessel. It may be used more than once.

Glasses are removed the next morning and cleansed in running tap water until the yellow color disappears. They are then placed in ammonia alcohol until used. When used wipe with soft, clean linen or cotton cloth. If old cover-slips are used, boil first in 5 per cent. sodium carbonate solution.

Another procedure is, after washing with soap and water and rinsing in water, to soak the cover-glasses in alcohol, then wipe with soft linen, then place in a Petri dish, and heat in the dry sterilizer for one hour at 200° C. to burn off fatty substances. The heating may be done by holding the cover-glass in the flame sufficiently to heat thoroughly without softening. A cover-glass is not clean when a drop of water spread over it does not remain evenly distributed, but gathers in droplets.

of the growth is diluted by adding it to a tiny drop of filtered or distilled water, free from all suspended matter, which has been previously placed on the glass. The amount of dilution is learned after a few trials. It is best to add to the drop just enough of the culture to make a perceptible cloudiness. The mixture is then smeared thinly and uniformly over the glass. When blood or pus is to be studied it is well to put a small drop on a slide or cover-glass and then immediately to place on top of this another slide or cover-glass. The fluid will spread between the two, and when they are drawn apart a fairly thin, even smear will be left on each of them. If it is desired to preserve the blood cells intact the films are placed in a saturated solution of corrosive sublimate for two or three minutes and then washed in running water, or they may be exposed to the vapor of formalin, or be placed in methyl alcohol or absolute ethyl alcohol for a few seconds before staining.

Milk films, after fixation, are cleared of fat by means of ether or alkaline solutions.[1] From whatever source derived the film is allowed to dry thoroughly at the usual air temperature, and then, in order to fix the film with its contained bacteria to the glass, the latter is grasped in any one of the several kinds of forceps commonly used, and is passed three times by a rather slow movement through the Bunsen or alcohol flame. Instead of this method the film may be fixed to the glass before becoming completely dried by placing it in any one of the already named fixatives for a few minutes. The smear thus prepared is usually stained either by the simple addition of a solution of an aniline dye, for from a few seconds to five minutes, or by one of the more complicated special stains described later. When the stain is to be hastened or made more intense the dye is used warm. For ordinary staining, the bacteria are simply covered completely by the cold staining fluid, which is left the requisite length of time.

The cover-glass or slide, with the charged side uppermost, may either rest on the table or be held by some modification of Cornet's forceps. When the solution is to be warmed the cover-glass may be floated, smeared side down, upon the fluid contained in a porcelain dish resting on a wire mat, supported on a stand, or the solution may be poured on the glass which may then be held over the flame in the Cornet forceps. If a slide is used it is simply inserted in the fluid or covered by it. The fluid both in the dish and on the glass should be carefully warmed so as to steam without actually boiling. The glass should be kept completely covered with fluid.

The bacteria having now been stained, the cover-glass or slide is grasped in the forceps and thoroughly but gently washed in clean water and then dried, first between layers of filter-paper and then in the air or high over a flame. A drop of balsam or water is then placed on a glass slide and the cover-glass put upon in with the bacterial side down. The cover-glass or slide preparation is now ready for microscopic examination after the addition of a drop of oil.

Stains Used for Bacteria.—The protoplasm of mature bacteria

[1] One-half to one per cent. sodium hydrate.

reacts to stains much as nuclear chromatin, though sometimes more and sometimes less actively.

Though bacteria may be stained with various dyes of very different chemical composition, such as hæmatoxylin and certain plant dyes, the best stains are the basic aniline dyes, which are compounds derived from the coal-tar product aniline ($C_6H_5NH_2$).[1] R. Koch was the first to recognize the affinity of bacteria for these dyes and to note their importance as a means of differentiating microörganisms from other corpuscular elements.

Aniline Dyes.—The aniline dyes which are employed for staining purposes are divided into two groups according as the staining action depends on the basic or the acid portion of the molecule. The former contain amido groups and are spoken of as nuclear stains, since they color the nuclear chromatin of both cells and bacteria. The latter contain hydroxyl groups and stain bacteria faintly; they are used chiefly for contrast coloring. The basic dyes are usually employed as salts of hydrochloric acid, while the acid dyes occur as sodium or potassium salts.

The following are the most commonly used basic aniline stains:

Violet stains—methyl violet, gentian violet, crystal violet.
Blue stains—methylene blue, thionin blue.
Red stains—basic fuchsin, safranin.
Brown stain—Bismarck brown.
Green stain—methyl green.

Of the above stains the violet and red stains are the most intense in action. It is correspondingly easy to overstain a specimen with them. Of the blue, methylene blue probably gives the best differentiation of structure and it is difficult to overstain with it.

These dyes are all more or less crystalline powders, and while some are definite chemical compounds, others are mixtures. For this reason various brands are met with on the market and the exact duplication of stains is not always possible. Dyes should be obtained from reliable houses only; most bacteriologists obtain them from Grübler, of Leipzig.

It is advisable to keep on hand not only the important dyes, but also stock solutions from which the staining solutions are made. The stock saturated alcoholic solutions are made by pouring into a bottle enough of the dye in substance to fill it to about one-quarter of its capacity. The bottle should then be filled with alcohol, tightly corked, well shaken, and allowed to stand twenty-four hours. If at the end of this time all the staining material has been dissolved, more should be added, the bottle being again shaken and allowed to stand for another twenty-four hours. This must be repeated until a permanent sediment of undissolved coloring matter is seen upon the bottom of the bottle. This bottle will then be labeled "saturated alcoholic solution," of whatever dye has been employed. The alcoholic solutions are not themselves

[1] For a good description of the composition and action of the various stains see A. B. Lee's "Microtomist's Vade-Mecum," 6th edition, 1905.

employed for staining purposes. The solution for use is made by filling a small bottle three-fourths with distilled water, and then adding the concentrated alcoholic solution of the dye, little by little, until one can just see through the solution. It is sometimes desirable to use a more concentrated solution with dyes such as methylene blue. Care must be taken that the color does not become too dense; usually about one part to ten is sufficient. Small wooden cases come prepared for holding about one-half dozen bottles of the staining solutions. This number will answer for all practical purposes.

General Observations on the Principles of Staining Bacteria.—The staining of bacteria is not to be considered simply as a mechanical saturation of the cell body with the dye, in which the latter is dissolved in the plasma. It is rather a chemical combination between the dye substance and the plasma. This union, however, is apparently an unstable one and easily broken up. Unna believes that the basic aniline dyes, from their chemical composition, are not really bases, but neutral salts—*e. g.*, fuchsin equals rosaniline chloride; they are called basic only because the staining components (as the rosaniline) are of a basic nature. The staining process is, therefore, not to be looked upon as if the dye substance separated into its component parts and only the staining ingredient attacked the cell body, because the tissues for which these "basic aniline dyes" have special affinity are themselves basic. On the contrary, the dyestuff unites as a whole with the plasma, forming, as it were, a double salt or unstable compound between the two.

The dependence of the staining process upon the solvent condition of the dye is shown in the following observations:

1. Entirely water-free, pure alcoholic dye solutions do not stain.

2. Absolute alcohol does not decolorize bacteria, while diluted alcohol is an active decolorizing agent. The compound of dye substance and plasma is therefore insoluble in pure alcohol.

3. The more completely a dye is dissolved the weaker is its staining power. For this reason pure alcoholic solutions are inactive; and the so-called weak dye solutions to which strong dye solvents have been added are limited in their action on certain bacteria in which the dye substance is closely united. This is the principle of Neisser's stain for diphtheria bacilli—viz., acetic acid methylene-blue solution.

On the other hand, the addition of alkalies to the dye mixture renders the solvent action less complete and the staining power more intense. According to Michaels, however, in Loeffler's methylene-blue solution the rôle of the alkali is purely of a chemical nature, by which it converts the methylene blue into methylene azure (azure II).

The dependence of the staining process upon the nature of the bacteria is exhibited in the following facts:

Certain bacteria stain easily, others with difficulty. To the latter belong, for example, the tubercle bacillus and lepra bacillus. Spores and flagella also stain with difficulty. The easily stained objects re-

quire but a minimum of time to be immersed in a watery solution, while the others must be stained by special dyes with or without the aid of outside influences (heat, mordants, etc.). The difficultly stained objects are at the same time not easily decolorized. The explanation of the resistance which these bacteria show to staining as well as to decolorizing agents is to be sought in two ways: either on the assumption that they possess a difficultly permeable or a resisting envelope, or that they have a special chemical constitution. The latter hypothesis holds good only, if at all, in regard to flagella and spores; while the assumption of the resisting envelope has reference more particularly to the tubercle bacillus, and is probably correct. The presence of fatty and waxy bodies in the envelope of these microörganisms is capable of demonstration. Moreover, after extraction of these bodies by ether the tubercle bacillus loses its power of resisting acids, which peculiar resistance can also be artificially produced in other bacteria having normally no such resisting power. In many instances, doubtless, both of these causes, viz., resistant envelope and chemically different constitution, work together to produce the above-mentioned results.

Individual differences in acid resistance among the difficultly stained bacteria have been observed in tubercle bacilli; according to Ziehl and Ehrlich, those having less individual resistance are probably the younger members. Individual differences in staining, in the easily stained bacteria, have also been noticed; for example, cholera vibrios and allied species are best stained with fuchsin, not so well with methylene blue, etc.

The relation between staining and degeneration of bacteria is a complicated question. Decrease of staining power takes place during degeneration of the bacterial cell, but it is often difficult to determine the exact moment when this loss of power occurs. Degenerated forms of the cholera bacillus from the abdominal cavity of guinea-pigs thus soon lose their power of staining in methylene-blue solution, but stain well in diluted carbol fuchsin. Moreover, bacteria killed by drying and moderate heating, as in the preparation of films, retain their power of staining. Kitasato found dead tubercle bacilli in sputum which took on normal staining. Bacteria killed by chloroform, formalin, etc., still retain their staining properties intact.

Elective staining properties, whereby certain species of bacteria are exclusively or rapidly and intensely stained by certain dyes, have repeatedly been observed. Of the greatest practical importance in this respect is the *Gram stain* (see p. 33, and Chap. XVI), used for the differential diagnosis of many species of bacteria; although a distinct classification of bacteria into those which are stained and those which are not stained by Gram's solution has been shown to be impracticable. There are some bacteria, however, which act uniformly toward Gram under all conditions; as, for example, the anthrax bacillus and the pyogenic cocci are always positive, the cholera and plague bacilli and gonococci are always negative to Gram. Other species again are

at one time stained and at another decolorized by Gram; thus pyocyaneus is stained only in young individuals. Previous heating or extraction with ether does not prevent the action of Gram's stain, but treatment with acids or alkalies renders it impossible. Bacteria so treated, however, after one hour's immersion in Loeffler's mordant regain their property of staining with Gram.

As to the nature of Gram's staining solution, it may be mentioned that only the pararosanilines (gentian violet, methyl violet, and Victoria blue) are suitable for the purpose, whereas the rosanilines (fuchsin and methylene blue) give negative results. The reason for this is that the iodine compounds with the pararosanilines are fast colors, while those with the rosanilines are unstable. These latter compounds when treated with alcohol break up into their constituents, the iodine is washed out, and the dye substance remaining in the tissues stain them uniformly; that is, without differentiation. But iodine-pararosaniline compounds are not thus broken up and consequently stain those portions of the tissue more or less, according to the affinity which they have for the dye substance. The parts stained by Gram are thus distinguished from those stained violet, not only quantitatively, but qualitatively; it is not a gentian violet, but an iodine-pararosaniline staining which occurs.

Use of Mordants and Decolorizing Agents.—We have already noted that the protoplasm of unrelated bacteria may respond differently to the several dyes. There is, however, seldom any difficulty in selecting a dye which will stain sufficiently to make bacterial cells in pure cultures distinctly visible. When the bacteria are imbedded in tissue or mixed in a film with blood or pus, it is frequently difficult to prevent the stain from so acting on the tissue or pus elements as to obscure the bacteria. Various methods are then employed to stain the bacteria more intensely than the tissues or to decolorize the tissue more than the bacteria. Heating, the addition of alkali to the staining fluid and prolonging the action of the dyes increase the staining of the bacteria. We regulate these so as to give the best results. We also use mordants; that is, substances which fix the dye to the bacterial cell, such as aniline oil or solutions of carbolic acid and metallic salts. As decolorizing agents we use chiefly mineral acids, vegetable acids, diluted alcohols, and various oils.

Formulæ of the Most Commonly Used Stain Combinations.—LOEFFLER'S ALKALINE METHYLENE-BLUE SOLUTION.—This consists of concentrated alcoholic solution of methylene blue, 30 c.c.; caustic potash in a 0.01 per cent. solution, 100 c.c. The alkali not only makes the cell more permeable, but also increases the staining power by liberating the free base from the dye.

KOCH-EHRLICH ANILINE-WATER SOLUTION OF FUCHSIN OR GENTIAN VIOLET is prepared as follows: To 98 c.c. of distilled water add 2 c.c. aniline oil, or, more roughly but with equally good results, pour a few cubic centimeters of saturated aniline oil into a test-tube, then add sufficient water nearly to fill it. In either case the mix-

tures are thoroughly shaken and then filtered into a beaker through moistened filter-paper until the filtrate is perfectly clear. To 75 c.c. of the filtrate (analine oil water) add 25 c.c. of the concentrated alcoholic solution of either fuchsin, methylene blue, or gentian violet, or add the alcoholic solution until the aniline water becomes opaque and a film begins to form on the surface.

CARBOLIC-FUCHSIN, OR ZIEHL-NIELSEN SOLUTION—Distilled water, 100 c.c.; carbolic acid (crystalline), 5 gm.; alcohol, 10 c.c.; fuchsin, 1 gm.; or it may be prepared by adding to a 5 per cent. watery solution of carbolic acid the saturated alcoholic solution of fuchsin until a metallic lustre appears on the surface of the fluid. The carbolic acid, like the alkali, favors the penetration of the stain.

The last two methods, combined with heating, are used to stain spores and certain resistant bacteria as the tubercle bacilli and other "acid resisters," so that they retain their color when exposed to decolorizing agents.

Carbolic-methylene blue, first used by Kühne, consists of 1.5 gm. of methylene blue, 10 gm. of absolute alcohol, and 100 c.c. of a 5 per cent. solution of carbolic acid. *Carbolic-thionin* consists of 10 parts of a saturated alcoholic solution of thionin and 100 parts of a 1 per cent. solution of carbolic acid.

GRAM'S STAIN.—Another differential method of staining which is employed is that known as Gram's method. In this method the objects to be stained are floated on or covered with the aniline or carbolic gentian-violet solution described above. After remaining in this for a few minutes they are rinsed in water and then immersed in an iodine solution (Lugol's), composed of iodine, 1 gm.; potassium iodide, 2 gm.; distilled water 300 c.c. In this they remain for from one to three minutes and are again rinsed in water. They are then placed in strong alcohol until most of the dye has been washed out. If the cover-glass as a whole still shows a violet color, it is again treated with the iodine solution, followed by alcohol, and this is continued until no trace of violet color is visible to the naked eye. It may then be washed in water and examined, or before examination it may be counter-stained for a few minutes by a weak solution of a contrasting dye, such as eosin, fuchsin, carmine, or Bismarck brown. This method is useful in demonstrating the capsule which is seen to surround some bacteria—particularly the pneumococcus—and also in differentiating between varieties of bacteria, for some do and others do not retain their stain when put in the iodine solution for a suitable time (see Chap. XVI, for further remarks upon Gram's stain).

Staining of Capsules.—Many methods of demonstrating the capsule have been devised. Two only will be given here.

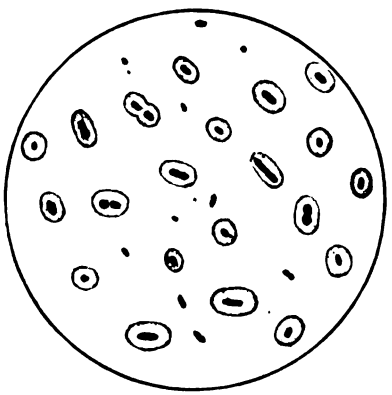
Welch's glacial acetic acid method is as follows: 1. Cover the preparation with glacial acetic acid for a few seconds. 2. Drain off and replace with aniline gentian-violet solution; this is to be repeatedly added until all the acid is replaced. 3. Wash in 1 to 2 per cent.

solution of sodium chloride and mount in the same. Do not use water at any stage. The capsule stains a pale violet.

HISS' COPPER SULPHATE METHOD (Fig. 18).—The organisms are grown, if possible, on ascitic fluid or serum media. If not, the organisms should be spread on the cover-glass mixed with a drop of serum, or, better, with a drop of one of the diluted serum media. Dry in the air and fix by heat.

The capsules are stained as follows: A 5 per cent. or 10 per cent. aqueous solution of gentian violet or fuchsin (5 c.c. saturated alcoholic solution gentian violet to 95 c.c. distilled water) is used. This is placed on the dried and fixed cover-glass preparation and gently heated for a few seconds until steam arises. The dye is washed off with a 20 per cent. solution of copper sulphate (crystals). The preparation is then placed between filter-paper and thoroughly dried.

FIG 18



Capsule stain by Hiss' method. Rhinoscleroma bacillus. × 1000. (Thro.)

Staining Spores and Acid-fast Bacteria.[1]—We have already noted that during certain stages in the growth of a number of bacteria spores are formed which refuse to take up color when the bacteria are stained in the ordinary manner. Special methods have been devised for causing the color to penetrate through the resistant spore membrane. In the simplest method a coverslip after having been prepared in the usual way is covered with Ziehl's carbolic fuchsin solution and held over the Bunsen flame until the fluid steams. This is continued for one or two minutes. It is then washed and dipped in a decolorizing acid solution, such as a 2 per cent. alcoholic solution of nitric acid, or a 1 per cent. solution of sulphuric acid in water, until all visible color has disappeared, then it is washed and dipped for one-half minute in a saturated watery solution of methylene blue. The bodies of the bacilli are blue and the spores red. This same method is used for staining acid-fast bacilli. Sometimes the spores refuse to take the stain in this manner. We then can adopt *Moeller's method*, which is designed still further to favor the penetration of the coloring matter through the spore membrane. The prepared cover-slip is held for two minutes in chloroform, then washed off in water, and placed from one-half to three minutes in a 5 per cent. solution of chromic acid, again washed off in water, and now stained by carbolic fuchsin, which is steamed for several minutes. The staining fluid is then washed off and the preparation decolorized in a 3 per cent. solution of hydrochloric acid or a 5 per cent. solution of sulphuric acid. The

[1] Special staining methods for the individual organisms are given in Part II.

preparation is finally stained for a minute in methylene-blue solution. The spores will be red and the body of the cells blue. The different spores vary greatly in the readiness with which they take up the dyes, and we have, therefore, to experiment with each variety as to the length of time it should be exposed to the maceration of the chromic acid. Even under the best conditions it is almost impossible to stain some spores.

Staining Flagella.—For the demonstration of flagella, which are possessed by all motile bacteria, we are indebted first to Loeffler. The staining of flagella satisfactorily is one of the most difficult of bacteriological procedures. Special stains devised by him, by Van Ermengem, by Pitfield, and others are employed. In all methods young (twelve-to eighteen-hour) cultures of agar should be chosen. Enough of the culture to produce slight cloudiness is placed in a few cubic centimeters of filtered tap water in a test-tube. This may be used immediately, or allowed stand in the thermostat at blood heat for from one to two hours to permit slight development. A tiny drop of this rather thin emulsion is allowed to spread with as little manipulation as possible over the cover-glass so that it may dry quickly. This latter point seems to be the important one since slow drying allows the bacteria to shed their flagella. We have gotten very good results by placing on the cover-glass with considerable force the tiny drop held in the platinum loop, in order to spatter extremely tiny drops which may dry in a minimum of time.

Bunge's modification of Loeffler's method is carried out as follows: Cover-glasses which have been most carefully cleaned are covered by a very thin smear. After drying in the air and passing three times through the flame the smear is treated with a mordant solution, which is prepared as follows: To 3 parts of saturated watery solution of tannin add 1 part of a 25 per cent. solution of ferric chloride. This mordant should be allowed to stand for several weeks before using. After preparing the cover-slip with all precautions necessary to cleanliness, the filtered mordant is allowed to act cold for five minutes, after which it is warmed and then in one minute washed off. After drying, the smear is stained with the carbol-fuchsin solution or carbol-gentian violet, and then washed, dried, and mounted.

Frequently the flagella appear well stained, but often the process has to be repeated a number of times. Overheating of the film prevents the staining of the flagella. The cell membrane may also show by this method.

Van Ermengem's method gives good results. It is as follows: The films are placed for one hour at room temperature, or are heated for five minutes over a water-bath at 100° C. in the following solution:

SOLUTION A.

Osmic acid, 2 per cent. solution ..... 1 part.
Tannin, 10 to 25 per cent. solution..... 2 parts.

Wash successively with water, absolute alcohol, and water, then place in the following solution for a few seconds:

SOLUTION B.

0.5 per cent. solution of $AgNO_3$ in distilled water.

Without washing transfer them to a third solution:

SOLUTION C.

Gallic acid	5 grms.
Tannin	3 grms.
Fused potassium acetate	10 grms.
Distilled water	350 c.c.

After keeping in this for a few seconds, place again in solution B until film begins to turn black. Then wash and examine.

Examination of Bacteria in Tissues.—Occasionally it is of importance to examine the bacteria as they occur in the tissues themselves. The tissues should be obtained soon after death, so as to prevent as much as possible post-mortem changes, with consequent increase or decrease in the number of bacteria. Selected pieces of tissues can be frozen by ether or carbon dioxide and sections cut, but the best results are obtained when the material is embedded in paraffin or in celloidin. From the properly selected spots small portions, not larger than one-quarter of an inch by one-eighth inch, are removed and placed in absolute alcohol for from four to eight hours, and longer if thicker. For the larger pieces it is better to change the alcohol after eight hours. The pieces of tissue should be kept from falling to the bottom as the higher layers of alcohol remain nearer absolute. If along with the bacteria one wishes to study the finer structure of the tissue, it is better to employ another fixative, formalin or corrosive sublimate. Corrosive sublimate (saturated solution in 0.75 per cent. sodium chloride solution) is an excellent fixing agent. Dissolve the sublimate in the salt solution by heat, allow it to cool; the separation of crystals will show that saturation is complete. For pieces of tissue one-eighth inch in thickness four hours' immersion is sufficient, for larger, twenty-four hours may be necessary. They should then be placed in pieces of gauze and left in running water for from twelve to twenty-four hours, according to the size of the pieces, to wash out the excess of sublimate. They are then placed successively for twenty-four hours each in the following strengths of methylated spirit (free from naphtha): 30 per cent., 60 per cent., and 90 per cent. Finally they are placed in absolute alcohol for twenty-four hours and are then ready to be embedded in paraffin (see Sec. III). The paraffin sections of tissue having been prepared and cut, they are ready for staining. If all of the sublimate has not been removed by the water the sections may be immersed in iodine-alcohol for ten minutes. For fixing in formalin the tissue is put in 4 to 10 per cent. formalin solution for three to twenty-four hours, and then in the alcohols.

LOEFFLER'S STAINING METHOD.—The section is placed in Loeffler's alkaline methylene-blue solution for 5 to 30 minutes, then placed for a few seconds in 1 per cent. acetic acid. It is then placed in absolute alcohol, xylol, and Canada balsam. The number of seconds during which the preparation remains in the acetic acid must be tested by trials.

The bacteria should be dark blue, the nuclei blue, and the cell bodies light blue.

Thionin solution, carbol-fuchsin solution, and gentian violet can be used instead of Loeffler's methylene blue. Gram's method, with 3 per cent. hydrochloric acid in alcohol as a tissue decolorizer for ten seconds, is also valuable.

Preservation of Specimens.—Dry stained preparations of bacteria keep indefinitely, but if mounted in Canada balsam, cedar oil, or dammar lac they tend gradually to fade, although many preparations may be preserved for many months or years. Dry unstained spreads should be kept in the ice-box until stained.

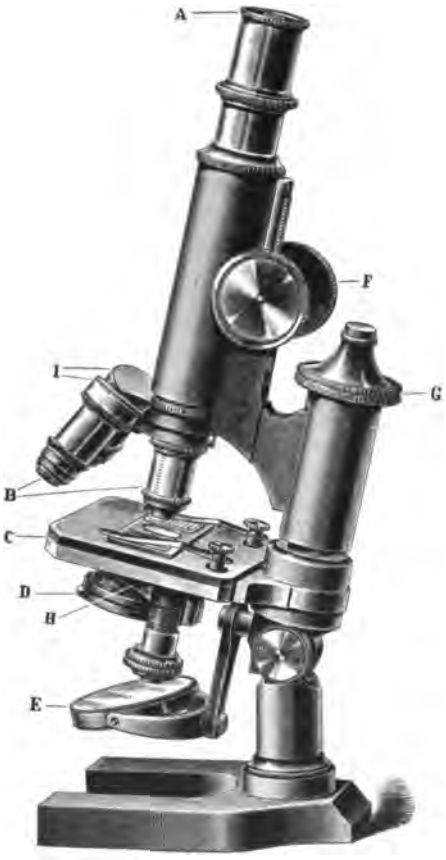
THE MICROSCOPE AND THE MICROSCOPIC EXAMINATION OF BACTERIA.

Different Parts of the Microscope (Figs. 19 and 20).—A complete instrument usually has four oculars, or eye-pieces (*A*), which are numbered from 1 to 4, according to the amount of magnification which they yield. Nos. 2 and 4 are most useful for bacteriologic work. The objective—the lens at the distal end of the barrel (B)—serves to give the main magnification of the object. For stained bacteria, the $\frac{1}{12}$ achromatic oil-immersion lens is regularly employed; for photographic purposes the apochromatic lenses are needed, although even here they are not indispensable. A $\frac{1}{16}$ lens may at times be useful, but hardly necessary; a No. 4 ocular and a $\frac{1}{12}$ lens give a magnification of about 1000 diameters (Fig. 21). For unstanied bacteria we employ either the $\frac{1}{12}$ immersion or $\frac{1}{7}$ dry lens, according to the purpose for which we study the bacteria; for the examination of colonies where, as a rule, we do not wish to see individual bacteria, but only the general appearance of whole groups, we use lenses of much lower magnification (Fig. 22).

The stage *C*—the platform upon which the object rests—should be large enough to support the Petri plates if culture work is to be done. The distance between the optical axis of the instrument and the pillar must be great enough to permit one to examine rather more than half the surface of the Petri dish without revolving it. The iris diaphragm *D*, which is now regularly used in bacteriologic work, opens and closes similar to the iris of the eye, and so controls the amount of light. Its opening is diminished or increased by moving a small arm, which is underneath the stage, in one or another direction. The reflector or mirror (E) placed beneath the stage serves to direct the light to the object to be examined. It has two surfaces—one concave and one plane. The concave surface must not be employed when the substage condenser is being used, otherwise the rays of light reaching the stage from the condenser will not be correctly focused. The concave surface may be used when unstained objects, such as colonies, hanging drops, are examined. At the same time the Abbe condenser should be lowered and the iris blender regulated. The coarse adjustment

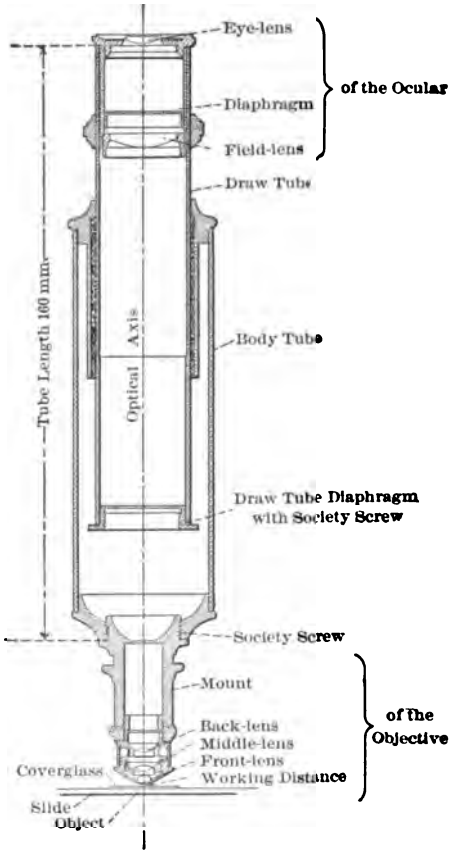
F is the rack-and-pinion arrangement by which the barrel of the microscope can be quickly raised or lowered. It is used to bring the bacteria roughly into focus. If the bearings become loose tighten the little screws at the back of the pinion box. Keep the teeth clean. If the bearings need oiling use an acid-free lubricant, such as paraffin oil. The fine adjustment *G* serves to raise and lower the barrel very slowly and evenly, and is used for the exact study of the bacteria when

FIG. 19.



Microscope.

FIG. 20.

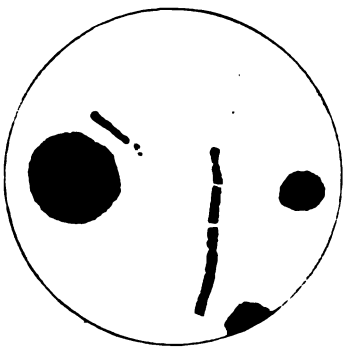


Internal structure of the microscope.

high-power lenses are used. It is necessarily of limited range and delicate in its mechanism. If, when looking into the eye-piece, no change of focus is noticed by turning the micrometer head, or if the micrometer head ceases to turn, the adjustment has reached its limit. Raise the barrel of the microscope by means of the coarse adjustment, then turn the micrometer back to bring the fine adjustment midway within its range. When the fine adjustment head stops do not force it. For the microscopic study of bacteria it is essential that we magnify

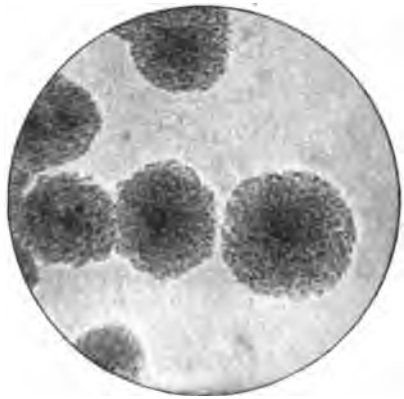
the bacteria as much as possible and still have their definition clear and sharp. For this purpose the microscope should be provided with an oil-immersion system and a substage condensing apparatus. In using the oil-immersion lens a drop of oil (oil of cedar) of the same index of refraction as the glass is placed upon the face of the lens, so as to connect it with the cover-glass when the bacteria are in focus. There is thus no loss of sight through deflection, as is the case in the dry system. If the lenses become dirty they should be wiped gently with Japanese lens paper or a clean, soft, old-linen handkerchief.

FIG. 21



Anthrax bacilli and blood cells.
× 1000 diameters.

FIG. 22



Colonies of diphtheria bacilli.
× 200 diameters.

If necessary breathe on the lens before wiping, and if this does not succeed use a little xylol or chloroform. These substances are not to be used unless necessary. An immersion objective should always be cleaned immediately after using. The objective should always be kept covered so as to prevent dust dropping in.

Light.—The best light is obtained from white clouds or a blue sky with a northern exposure. Avoid direct sunlight. If necessary use white shades to modify the sunlight. Artificial light has one advantage over daylight in that it is constant in quality and quantity. The Welsbach burner and a whitened incandescent bulb give a good light. A blue glass between the artificial light and the lens is often of value. An eye-shade may be helpful.

Substage Condensing Apparatus *H* is a system of lenses situated beneath the central opening of the stage. It serves to condense the light passing through the reflector to the object in such a way that it is focused upon the object, thus furnishing the greatest amount of luminosity. Between the condenser and the reflector is placed the iris diaphragm.

Focusing.—Focus the body tube down by means of the coarse adjustment until the objective approaches very near to the cover-

glass, being careful not to touch it. Then with the eye at the eye-piece focus up carefully with the coarse adjustment until the specimen comes plainly into view. Be careful not to pass by this focal point without noticing it. This is likely to occur if the light be too intense and the specimen thin and transparent. If the sliding tube coarse adjustment is used, focus carefully by giving the tube a spiral movement.

When the object is brought fairly well into focus by means of the coarse adjustment, use the fine adjustment to focus on the particular spot desired, for if this spot is in the centre of the field of the low power it should be somewhere in the field of the higher power. It is too much to ask of the maker that the lenses be made absolutely parfocal and centred. The delicacy of the centring can be appreciated when the magnification and the extremely small portion examined are considered. When the objectives are not thus fitted to the nose-piece, refocusing and again hunting up the object are necessary. In so doing we repeat the caution always to focus up before turning the nose-piece. When no revolving nose-piece is used the change of objectives means the unscrewing of one and the screwing of the other into its place, and refocusing.

The beginner should always use the low-power objectives and oculars first. The low-power objectives have longer working distances and are not so apt to be injured. They always show a larger portion of the specimen and thus give one a better idea of the general contour. After obtaining this general idea the higher powers can be used to bring out greater detail in any particular part. Generally speaking, it is best to use a high-power objective and low-power eye-piece in preference to a low-power objective and high-power eye-piece. In the latter case any imperfections in the objective are magnified unduly by the eye-piece, giving, as a rule, poor definition.

Tube Length and Cover-glass.—All objectives are corrected to a certain tube length (160 mm. by most makers—Leitz, 170 mm.) and all objectives in fixed mounts of over 0.70 N. A. are corrected to a definite thickness of cover-glass as well (Zeiss, 0.15 mm., 0.20 mm.; Leitz, 0.17 mm.; Bausch & Lomb and Spencer, 0.18 mm.). These objectives give their best results only when used with the cover-glass and tube length for which they are corrected. As indicated in Fig. 53 the tube length extends from the eye lens of the eye-piece to the end of the tube into which the objective or nose-piece is screwed. If a nose-piece is used the draw tube must be correspondingly shortened. If the cover-glass is thinner than that for which the objective is corrected, the tube must be lengthened to obtain best results; if thicker, shortened.

The more expensive objectives are provided with adjustable mounts by which the distances between the lens systems may be changed to compensate for difference of thickness of cover. They are successfully used only in the hands of an expert. One of them out of adjustment is worse than an ordinary objective.

Examination of Bacteria in the Hanging Drop.—As we stated at the beginning of this chapter, it is often valuable to observe bacteria alive, so as to study them under natural conditions. We can thus note the method and rate of their multiplication, the presence or absence of spore formation, and their motility in fluids and their agglutination with specific serums. For this examination special slides and methods are desirable. The slide used is one in which there is ground out on one surface a hollow having a diameter of about half an inch (Fig. 23). According to the purpose for which the hanging drop is to be studied, sterilization of the slide and cover-glass may or may not be necessary. The technique of preparing and studying the hanging drop is as follows: The surface of the glass around the hollow in the slide is smeared with a little vaselin or other inert substance. This has for its purpose both the sticking of the cover-glass to the slide

FIG. 23

Hollow slide with cover-glass.

and the prevention of evaporation in the drop placed in the little chamber, which is to be formed between the cover-glass when placed over the hollow, and the slide.

For the purpose of studying the bacteria we place, if they are in fluids, simply a large platinum loopful upon the centre of the cover-glass and, to avoid drying, immediately invert it by means of a slender pair of forceps over the hollow in the slide, being very careful to have the drop over the very centre of the cover-glass. The cover-glass is then pressed on the slide so as to spread the vaselin and make a perfect seal. If the bacteria, on the contrary, are growing on solid media, or are obtained from thick pus or tissues from organs, they are mixed with a suitable amount of bouillon or sterile physiological salt solution either before or after being placed upon the cover-glass. If we wish to observe the bacteria under natural conditions we must keep the tiny drop of fluid at the proper temperature for the best growth of the bacteria. If, however, we simply wish to observe their form and arrangement this is not necessary.

In the study of living bacteria we often wish to observe their grouping and motion rather than their individual characters, and so use less magnification than for stained bacteria. In studying unstained bacteria and tissues we shut off as large a portion of the light with our diaphragm as is compatible with distinct vision, and thus favor contrasts which appear as lights and shadows, due to the differences in light transmission of the different materials under examination. It is necessary to remember that they are seen with difficulty, and that we are very apt, unless extremely careful in focusing, to allow the lens to go too far, and so come upon the cover-glass, break it, destroy our preparation, and, if examining parasitic bacteria, infect the lens.

This may be avoided by first finding the hanging drop with a low-power lens and thus exactly centre it. The lens of higher magnification is now very gradually lowered, while at the same time gently moving the slide back and forth to the slightest extent possible with the left hand. If any resistance is felt the lens should be raised, for it has gone beyond the point of focus and is touching the cover-glass.

Hanging Mass or Hanging Block Cultures.—In order to study the morphology and manner of multiplication of bacteria to better advantage than in the hanging drop, we have used hanging masses of agar, made by placing a large platinum loop full of melted agar on a sterile cover-glass and allowing it to harden, protected from dust. The bacteria are placed on the free surface of this mass which is then inverted over a hollow slide and studied as in a hanging drop.

Hill devised the following procedure: Melted nutrient agar is poured into a Petri dish to a depth of about one-eighth to one-quarter of an inch. When cool a block is cut out about one-quarter of an inch square. The block is placed, under surface down, on a slide and protected from dust. A very dilute suspension of the growth to be examined is then made in sterile bouillon and spread over the upper surface of the block. The slide and block are then put in the incubator for ten minutes to dry slightly. A clean cover-slip is now placed on the agar block in such a way as to avoid large air bubbles. The slide is then removed. With the aid of a platinum loop a drop or two of melted agar is run along each side of the block to fill any angles between it and the cover-glass. After drying in the incubator for five minutes it is placed over a hollow slide and sealed with paraffin.

We consider the hanging mass method better than that of the hanging block in many instances, because in the former method no pressure is exerted on the bacteria, and more oxygen is allowed them.

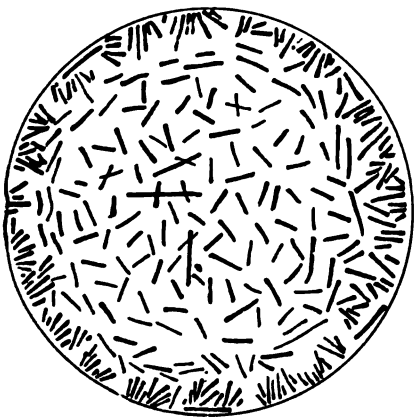
Testing Agglutinative Properties of Serum.—By agglutination is meant the aggregation into clumps of uniformly disposed bacteria in a fluid; by sedimentation, the formation of a deposit composed of such clumps when the fluid is allowed to stand; sedimentation is thus the naked-eye evidence of agglutination.

The blood serum of animals is found to acquire the clumping power for almost every variety of motile bacteria, and for many non-motile forms after infection with each variety. The substances causing the clumping are called *agglutinins*. (For a discussion of agglutination see later chapter.)

The agglutinins were discovered by Gruber and Durham. Their effect on bacteria can be observed either macroscopically or microscopically. For example, if a serum from an animal which has passed through a typhoid infection is added to a twenty-four-hour culture of typhoid bacilli, and the mixture placed in a thermostat, the following phenomenon will be noticed: The bacteria, which previously clouded the bouillon uniformly, clump together into little masses, settle to the sides of the test-tube, and gradually fall to the bottom until the fluid is almost entirely clear. In a control test, on the contrary, to which

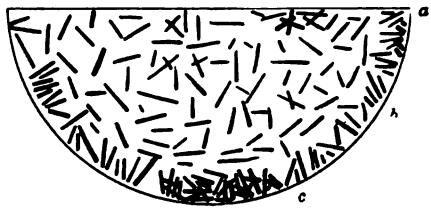
no active serum is added the fluid remains uniformly cloudy. The reaction is completed in from one to twelve hours. If the reaction is observed in a hanging drop, a gradual formation of clumps is seen. Frequently one sees bacteria which have recently joined a group make violent motions as though they were attempting to tear themselves away; then they gradually lose their motility completely. Even the larger groups of bacteria may exhibit movement as a whole. After not more than one or two hours the reaction is completed; in place of the bacteria moving quickly across the field, one sees one or several groups of absolutely immobile bacilli. Now and then in a number of preparations one sees a few separate bacteria still moving about among the groups. If the reaction is feeble, either because the immune serum has been highly diluted or because it contains very little agglutinin, the groups are small and one finds comparatively many isolated and perhaps also moving bacteria. It is essential each time to make a control test of the same bacterial culture without the addition of serum. Under some circumstances the reaction proceeds with extraordinary rapidity, so that the bacilli are clumped almost imme-

FIG. 24



Microscopic field, showing the top of a hanging drop in a normal typhoid culture.

FIG. 25



Microscopic field, showing a cross-section of the drop in Fig. 24.

diately. By the time the microscopic slide has been prepared and brought into view, nothing is to be seen of any moving or isolated bacteria, and only by means of the control test is it possible to tell whether the culture possessed normal motility.

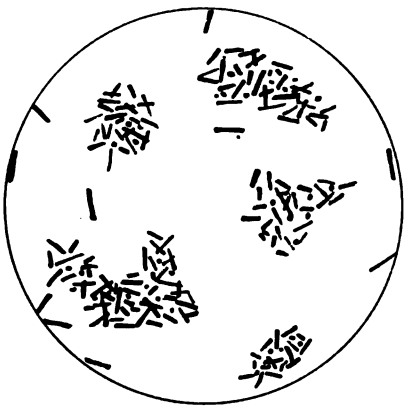
In order to help the student thoroughly to understand what comprises a reaction, Wilson prepared a set of drawings, which are here reproduced. The culture to be tested should be of about twenty hours' growth, either in bouillon or on agar. If on the latter a suspension is made in broth or normal salt solution. A loopful of the fluid containing the bacteria is placed on the cover-glass, and to it an equal quantity of the desired serum dilution is added.

In making the hanging drop to be examined it is necessary to have it of such a depth that it will show at least three focal planes, otherwise the examination will be incomplete and unsatisfactory. The moist chamber must be well sealed by vaselin so as to prevent drying, and kept at a temperature of at least 20° and not over 35° C.

Fig. 24 shows a microscopic field of the *top* of a hanging drop of a normal bouillon culture of typhoid bacilli. The culture is twenty hours old and the organisms are freely motile. This represents the control drop used for comparison with the drop of the same culture to which has been added a little of the blood of a person suspected to have typhoid. Note these points in Fig. 24; the organisms are evenly distributed throughout the field, except at the edge of the drop, where they are gathered in great numbers; they show great activity here, seemingly trying to crowd to the very edge. This attraction is probably due to the action exerted on the organisms by the oxygen in the air, which naturally exerts positive chemotaxis on all aërobic organisms.

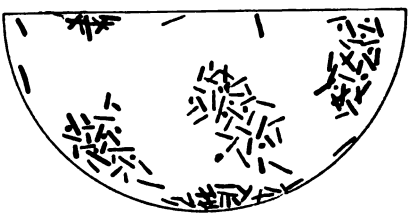
Fig. 25 shows a *cross-section* of the drop represented in Fig. 24, and it will be noticed that the bacilli are evenly distributed throughout the

FIG. 26



Microscopic field, showing the top of a drop with the typhoid reaction.

FIG. 27



Microscopic field, showing a cross-section of the drop in FIG. 26.

drop, except at one place in the focal plane *a*, and again in the focal plane *c*.

It sometimes happens that there is a substance adhering to a supposedly clean cover-glass which attracts the bacilli to that point, where they appear as fairly well-defined clumps, more or less like the true clumps due to the agglutinating substance in typhoid blood. The increase in organisms at the bottom of the drop in the focal plane *c* is easily accounted for by the fact that gravity naturally carries the dead and non-motile organisms to the bottom, these frequently assuming the character of clumps.

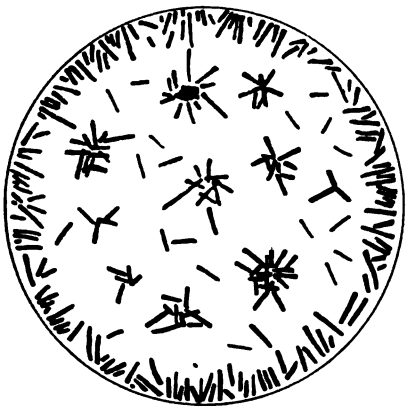
If a field can be found in any focal plane of the hanging drop free

from clumps, one can be quite sure that any clumping present is not due to any agglutinating substance which necessarily will affect organisms in every focal plane.

Fig. 26 shows the microscopic appearance of the *top* of a drop where the reaction is present. Notice first that the organisms have been drawn together in groups and that the individuals of each group appear to be loosely held together. Viewed under the microscope these clumps are practically quiescent, there being very little movement either of the individual organisms or of the clump as a whole. The edge of the drop is practically free from organisms, showing that the air no longer exerts any influence on them.

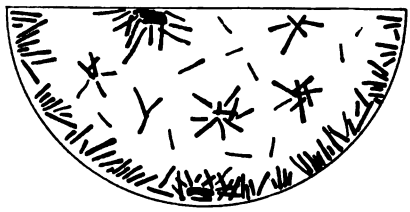
Fig. 27 shows a *cross-section* of the hanging drop shown in Fig. 26. The clumps are evenly distributed throughout the drop, with perhaps some increase in the numbers and compactness of the clumps at the bottom.

FIG. 28.



Microscopic field, showing the top of a drop of culture with reaction not due to typhoid.

FIG. 29.



Microscopic field, showing a cross-section of Fig. 28.

Fig. 28 shows the microscopic appearance of the *top* of a hanging drop of a bouillon culture to which has been added some blood of a patient suffering from a febrile condition not caused by typhoid infection, but which exerts a slight non-specific influence on the typhoid organisms. It will be seen that the reaction is incomplete and that there are many organisms at the edge of the drop. The air exerts the same influence on the bacilli that it did before the addition of the blood. Note the character of the clumps, generally small and compact at the centre, with the bacilli at the edge of the clump, usually attached by one end only.

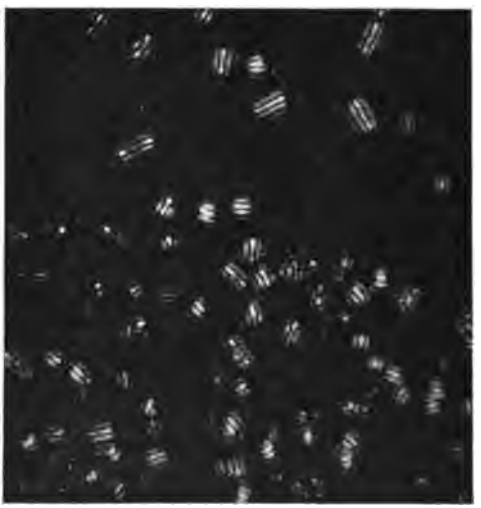
Very frequently these clumps have the appearance of being built up around a piece of detritus present in the clump. All the organisms comprising the clump seem to have retained part, at least, of their motility, those on the edges being particularly motile, so far as their free ends are concerned.

When motility is very much inhibited these clumps have a peculiar trembling movement, which is like the molecular movement described by Brown.

Fig. 29 shows a cross-section of the drop represented in Fig. 28. Note the same character of the clumps in every focal plane: the large number of motile bacilli and the number attracted at the edge of the drop by the air.

Dark Ground Illumination and the Examination of Ultramicroscopic Particles.—The apparatus constructed by Siedentopf and Zsigmondy[1] makes visible, and in solutions otherwise apparently homogeneous, very minute particles, which heretofore could not be seen even with the highest magnifications. Particles $1\mu\mu$ (a millimicron = one millionth of a millimeter) are thus rendered visible.

FIG. 30



Virulent diptheria bacilli. Cultures two days old. Unstained. × 2400. (After Siebert.)

This increased power in microscopic analysis is made possible by intense (electric arc lamp) focal lateral illumination of the objects examined, making them appear as minute luminous points. The greater the difference between the refractive index of the particles colloidally dissolved or otherwise held in suspension and the fluid which surrounds them, the brighter will be the appearance of the particles, and, therefore, the more readily visible.

The microscopic field, as will be seen by the photogram herewith (Fig. 30), is dark; the objects which refract the light show as brightly illuminated, sharply defined pictures, in which the black margin corresponds to the contour of the object. The illuminated portion is sur-

[1] Annalen der Physik, 4te Folge, Band 10.

rounded by a fine dark zone, this in turn by alternate bright and dark zones, in which the illumination rapidly decreases.

Reichert, of Vienna, has recently simplified this apparatus by devising a new condenser.[1] The light which illuminates the object has a greater refraction than the cone of light entering the objective which produces the image. Its advantages over the first method are: (1) It utilizes the source of light better; (2) any dry objective can be used without alterations; (3) small particles are seen without the disturbing refraction rings. With this apparatus such living organisms as the *Spirocheta pallida*, and the flagella on certain bacteria, which can scarcely be seen by ordinary microscopes on account of their low refractive indices, may be demonstrated with great clearness.

The use of *microphotography with ultra-violet light* (according to A. Kohler[2]) makes visible particles that cannot be seen by ordinary light, because of the inability of the violet rays to pass through certain substances, *e. g.*, chromatin. The few discoveries claimed by these means for diseases of unknown origin have so far lacked sufficient corroboration to constitute them proved.

Burri's India-ink method[3] of demonstrating bacteria. In 1907, 1908 and 1909 Burri recommended the following method for isolating and studying single bacterial cells. A solution of India ink (flüssige Perltusche) in water 1:10 [better 1–4] is sterilized in test-tubes in the autoclave for fifteen minutes. A small drop of this ink is mixed carefully with a drop of the fluid to be examined. If cultures from isolated cells are desired the fluid should first be diluted so that a drop contains *presumably* a single organism; then drops of the mixture are placed in rows upon nutrient agar plates. If the bacteria are to be examined immediately a drop of the mixture (ink plus undiluted bacterial fluid) is allowed to dry upon a glass slide and then examined under an oil-immersion lens. The bacteria appear a brilliant white upon a dark field, particles of the ink surrounding the organisms like a capsule. This method is especially applicable for the demonstration of such organisms as the *Tr. pallidum* which have poor staining qualities and a low index of refraction.

[1] Journ. R. Micr. Soc., 1907, p. 364, gives full description and instructions for use.

[2] A. Kohler. Ztschr. f. wiss. Mikroskopie, 1904, 21, 129.

[3] Burri, Robt. Das Tuscheverfahren als einfaches Mittel zur Lösung einiger schwieriger Aufgaben der Bakterioskopie, 1909. Jena, O. Fischer.

CHAPTER IV.

EFFECTS OF SURROUNDING FORCES UPON BACTERIA.

FOOD, OXYGEN, TEMPERATURE, LIGHT, ETC.

1. **Food.**—Naturally, the effect of food upon bacteria is marked. Though the majority of bacteria grow easily on certain artificial foods (culture media), some we have not yet been able to cultivate outside of the body of their host. Those bacteria which depend entirely upon a living host for their existence are known as *strict parasites;* those which live only upon dead organic (a few on inorganic) substances are called *strict saprophytes;* those which can lead a saprophytic existence, but which usually thrive only within the body of a living animal, are called *facultative parasites.* The *strict saprophytes,* which represent the larger majority of all bacteria, are not only harmless to living organisms, but perform many exceedingly important functions in nature, such as the destruction of dead organic matter and its preparation for plant food through decomposition, putrefaction, and fermentation, while one group (see below, the nitrifying bacteria) are constructive in their activities. The *parasites,* on the contrary, are harmful invaders of the body tissues, exciting by their growth and products many forms of disease. The substances essential for the majority of those forms which can be grown artificially are organic material as a source of carbon and nitrogen, an abundance of water and certain salts (either calcium or magnesium and sodium or potassium salts are usually required, also sulphur and phosphorus salts. Iron is demanded by a few varieties). The demands of bacteria for food of a certain composition vary considerably. The greater number of important bacteria and all the pathogenic species thrive best in media containing abundant albuminoid substances and of a slightly alkaline reaction to litmus. Some species of water bacteria, on the other hand, require so little organic material that they will grow in water that has been twice distilled. A certain species will grow abundantly in water containing ammonium carbonate in solution and no other source of carbon and nitrogen. Then there is a whole group of soil bacteria, the so-called nitrifying organisms which develop in the presence of very simple mineral salts (ammonium salts and nitrites). These show the power of some bacteria to produce cell substance from the simplest materials—a power formerly supposed to belong only to the higher plants which obtain their nourishment from the air through their chlorophyll and the assistance of sunlight. The bacteria, however, of any importance in disease are not so easily satisfied, though there are many species which are able to develop without

the presence of albumin and in comparatively simple culture media, such as the culture liquid proposed by Uschinsky, or the simpler one of Voges and Fraenkel, which consists of water, 1000; sodium chloride, 5; neutral sodium phosphate, 2; ammonium acetate, 6; and asparagin, 4. In these media many bacteria grow well.

When we consider in detail the source of the more important chemical ingredients of bacteria we find that their nitrogen is most readily obtained from diffusible albuminoid material and less easily from ammonium compounds. Their carbon they derive from albumin, peptone, and sugar, as well as from other allied carbohydrates: glycerin, fats, and other organic substances. It is an interesting fact that even compounds which in considerable concentration are extremely poisonous, can, when in sufficient dilution, provide the necessary carbon and even act as stimulants to growth; in this way carbolic acid in very dilute solutions may be used by some bacteria.

The value of substances as a source of nutrition is often influenced by the presence of other materials, as, for instance, the value of asparagin is increased by the presence of sugars. Further, materials from which nitrogen and carbon cannot be directly obtained still become assimilable after being subjected to the influence of bacterial ferments. The profound and diverse changes produced by the different ferments make it almost impossible to establish, except in the most general way, the nutritive value of any mixture for a large number of bacteria through a simple knowledge of its chemical composition.

The special culture media, such as bouillon, blood serum, etc., used for the development of bacteria, will be dealt with in a later chapter.

While it is true that very wide differences in relative composition and total concentration of food media may have slight effect upon the development of a given bacterium, slight changes in composition and reaction of the media often have a great effect upon morphology, rate of growth, motility, and specific products of growth.

Reaction of Media.—The reaction of the media is of very great importance. Most bacteria grow best in those media that are slightly alkaline or neutral to litmus. Only a few varieties require an acid medium, and none of these belong to the parasitic bacteria. An amount of acid or alkali insufficient to prevent the development of bacteria may still suffice to rob them of some of their most important functions, such as the production of poison. The different effect upon closely allied varieties of bacteria of a slight excess of acid or alkali is sometimes made use of in separating those which may be closely allied in many other respects.

Influence of One Species upon the Growth of Another.—When one species of bacteria is grown in a food medium, that medium usually becomes less suitable for the growth of its kind and of other bacteria. This is due partly to the impoverishment of the food stuffs, but more to the production of chemical substances or enzymes. When different species are grown together, the antagonistic action of one upon the

other may be shown from the beginning. Some species, however, have a coöperative or symbiotic action with other species.

In nature, bacteria usually occur in mixed cultures (*e. g.*, water, milk, intestinal contents of all animals), and here we may see antagonistic action in the prevalence of one species over others (*e. g.*, the lactic acid formers in the intestines), or coöperative action in the equal and luxuriant growth of two or more species (*e. g.*, pneumococcus and influenza bacillus in the lungs).

Experimentally, the existence of antagonisms can be demonstrated by inoculating gelatin streak cultures of various bacteria. It is found that many species will grow not at all or only sparingly when in close proximity to some other species. This antagonism, however, is often only one-sided in character. Again, when gelatin or agar plates are made from two different species of bacteria it may be observed that only one of the two grows. A third method of making this experiment is simultaneously to inoculate the same liquid medium with two species, and then to examine them later, both microscopically and by making plate cultures; not infrequently one species may take precedence of the other, which after a time it may entirely overcome.

Finally, it may be shown experimentally that bacteria may oppose one another as antagonists in the animal body. For instance, Emmerich has shown, that animals infected with anthrax may often be cured by a secondary infection with the streptococcus.

The symbiotic or coöperative action of bacteria may be demonstrated experimentally in the following examples:

a. Pneumococci when grown together with a bacillus obtained from the throat, produces very large, succulent colonies. The influenza bacillus which will not grow alone upon ordinary nutrient agar will grow well there in the presence of certain other bacteria. Some anaërobic species grow even with the admission of air if only some aërobic species are present (tetanus bacilli with diphtheria bacilli).

b. Certain chemical effects, as, for instance, the decomposition of nitrates to gaseous nitrogen, cannot be produced by many bacteria alone, but only when two are associated,

2. **Behavior toward Oxygen and other Gases.**—The majority of bacteria absolutely require free oxygen for their growth, but a considerable minority fail to grow unless it is excluded. This latter fact, noted first by Pasteur, led him to divide bacteria into aërobic and anaërobic forms. Between these two groups we have those that can grow either with or without the presence of oxygen, called respectively facultative aërobic and facultative anaërobic bacteria.

a. **Aërobic Bacteria.**—Growth only in the presence of free oxygen: the slightest restriction of air inhibits development. Spore formation, especially, requires the free admission of air.

b. **Anaërobic Bacteria.**—Growth and spore formation only in the total exclusion of free oxgyen. Among this class of bacteria are the bacillus of malignant œdema, the tetanus bacillus, the bacillus of symptomatic anthrax, and many soil bacteria. Exposed to the action

of oxygen, the vegetative forms of these bacteria are readily destroyed; the spores, on the contrary, are very resistant. Anaërobic bacteria being deprived of free oxygen—the chief source of energy supplied to the aërobic species, by which they oxidize the nutritive substances in the culture media—are dependent for their oxygen upon decomposable substances, such as grape-sugar.

c. **Facultative Aërobic and Facultative Anaërobic Bacteria.**—The greater number of aërobic bacteria, including most of the pathogenic species, are capable of withstanding, without being seriously affected, some restriction in the amount of oxygen admitted, and many, indeed, grow equally luxuriantly with the partial exclusion of oxygen. Life in the animal body, for example, as in the intestines, necessitates existence with diminished supply of oxygen. If in any given variety of bacteria, the amount of oxygen present is unfavorable, there will be more or less restriction in some of the life processes of this variety, such as pigment and toxin production, spore formation, etc. Pigment formation almost always ceases with the exclusion of oxygen, but poisonous products of decomposition may be more abundantly produced.

It is important to note that, according to recent investigations, it has been shown that the aërobic development of the anaërobes may be facilitated by the presence of living or dead aërobes.

It has also been observed not infrequently that certain species which on their isolation at first show more or less anaërobic development—that is, a preference to grow in the depth of an agar stick culture, for instance—after a while seem to become strict aërobes, growing only on the surface of the medium. This observation proves that the simple fact of an organism showing aërobic or anaërobic growth is not a sufficient basis for its separation into a distinct species.

Other Gases.—While all facultative bacteria as well as strict anaërobes grow well in nitrogen and hydrogen, they behave very differently toward carbonic acid gas. A large number of these species do not grow at all, being completely inhibited in their development until oxygen is again admitted—for example, *B. anthracis* and *B. subtilis* and other allied species. It has been found in some species, as glanders and cholera, that the majority of the organisms are quickly killed by CO_2, while a few, such as staphylococci, offer a great resistance, rendering impossible complete sterilization by means of this gas. Another group, again—viz., streptococcus and staphylococcus—exhibits a scanty growth. A mixture of one-fourth air to three-fourths carbonic acid gas seems to have no injurious effect on bacteria which cannot grow in an atmosphere of pure CO_2. Under pressure CO_2 is more effective (p. 56).

Sulphuretted hydrogen in large quantity is a strong bacterial poison, and even in small amounts kills some bacteria.

3. **Effect of Temperature upon Bacteria.**—Some form of bacterial life is possible within the limits of 0° and 70° C. The maximum and minimum temperature for each individual species ordinarily lies from 10° to 30° C. apart, and the optimum covers about 5°. Usually the temperature of the soil in which the bacteria are deposited is the con-

trolling factor in deciding whether growth will or will not take place. Thus, nearly all parasitic bacteria require a temperature near that of the body for their development, while many saprophytic bacteria can grow only at much lower temperatures. Bacteria when exposed to lower temperature than suffices for their growth, while having their activities decreased, are not otherwise injured unless actually frozen for a certain time; while exposure to higher temperatures than allows of growth more or less quickly destroys the life of the bacteria. Bacteria have been classified according to the temperatures at which they develop, as follows:

Psychrophilic Bacteria.—Minimum at 0° C., optimum at 15° to 20° C., maximum at about 30° C. To this class belong many of the water bacteria, such as the phosphorescent bacteria in sea-water.

Mesophilic Bacteria.—Minimum at 5° to 25° C., optimum about 37° C., maximum at about 43° C. To this class belong all pathogenic bacteria, most parasitic and many saprophytic forms.

Thermophilic Bacteria.—Minimum at 25° to 45° C., optimum at 50° to 55° C., maximum at 60° to 70° C. This class includes a number of soil bacteria which are almost exclusively spore-bearing bacilli. They are also found widely distributed in feces.

By carefully elevating or reducing the temperature the limits within which a variety of bacteria will grow can be altered. Thus, the anthrax bacillus was gradually made to accommodate itself to a temperature of 42° C., and pigeons, which are comparatively immune to anthraxl, partly on account of their high body temperature (42° C.), when inoculated with this anthrax succumbed to the infection. Another culture accustomed to a temperature of 12° C. killed frogs kept at 12° C. We have cultivated a very virulent diphtheria bacillus so that it will grow at 43° C. and produce strong toxin.

Effect of Low Temperature.—The rapidity of bacterial growth is retarded by temperatures lower than those required for the optimum of each species. It is the usual custom in laboratories to preserve bacteria which die readily (such as streptococci) by keeping them in the refrigerator at about 5° to 10° C., after cultivation for two days at 30° C., as a means for retaining their vitality without repeated transplantation. Temperatures even far under 0° C. are only slowly injurious to bacteria, different species being affected with varying rapidity. This has been demonstrated by numerous experiments in which they have been exposed for weeks in a refrigerating mixture at —18° C. If a culture of typhoid bacilli is frozen, about 50 to 70 per cent. of the organisms are killed at the time. At the end of one week not more than 10 per cent. survive, and at four weeks not over 1 per cent. After six months none survive. More resistant bacteria live longer and spores may survive in ice for years. Bacteria have even been subjected to a temperature of —175° C. by immersing them in liquid air kept in an open tube for two hours, and 15 to 80 per cent. were found still to grow when placed in favorable conditions. We found about 10 per cent. of typhoid bacilli alive after thirty minutes'

exposure to this low temperature. Staphylococci were more resistant. Spores were scarcely affected at all.

Effect of High Temperatures.—Temperatures from 5° to 10° C. over the optimum affect bacteria injuriously in several respects. Varieties are produced of diminished activity of growth, the virulence and the property of causing fermentation are decreased, and the power of spore formation is gradually lost. These effects may predominate either in one or the other direction.

If the maximum temperature is exceeded, the organism dies; the thermal death point for the psychrophilic species being about 37° C., for the mesophilic species about 45° to 55° C., and for the thermophilic species about 75° C. There are no non-spore-bearing bacteria which when moist are able to withstand a temperature of 100° C. even for a few minutes. A long exposure to temperatures between 60° and 80° C. has the same result as a shorter one at the higher temperatures. Ten to thirty minutes' exposure to moist heat will at 60° C. kill the cholera spirillum, the streptococcus, the typhoid bacillus, and the gonococcus, and at 70° C. the staphylococcus, the latter being among the most resistant of the pathogenic organisms which have no spores. A much shorter exposure will kill a large percentage of any mass of these bacteria.

Effect of Dry Heat.—When microörganisms in a desiccated condition are exposed to the action of heated dry air the temperature required for their destruction is much above that required when they are in a moist condition or when they are exposed to the action of hot water or steam. A large number of pathogenic and non-pathogenic species are able occasionally to resist a temperature of over 100° C. dry heat for from ten minutes to one hour. In any large number of bacteria a few are always more resistant than the majority. A temperature of 120° to 130° C. dry heat maintained for one and a half hours will destroy all bacteria, in the absence of spores.

Resistance of Spores to Heat.—Spores possess a great power of resistance to both moist and dry heat. Dry heat is comparatively well-borne, many spores resisting a temperature of over 130° C. for as long as three hours. Exposed to 150° C. for one hour, practically all spores are killed. Moist heat at a temperature of 100° C., either boiling water or free-flowing steam, destroys the spores of most varieties of bacteria within fifteen minutes; certain pathogenic and non-pathogenic species, however, resist this temperature for hours. The spores of a bacillus from the soil required five and a half to six hours' exposure to streaming steam for their destruction. They were destroyed, however, by exposure for twenty-five minutes in steam at 113° to 116° C. and in two minutes at 127° C. The spores from tetanus bacilli may require longer than fifteen minutes' exposure to kill them.

The resistance of spores to moist heat is tested by suspending threads, upon which the spores have been dried, in boiling water or steam. The threads are removed from minute to minute and laid upon agar

or in broth, which is then placed at a suitable temperature for growth, should any spores be living.

Practical Points on Heat Disinfection.—In the practical application of steam for disinfecting purposes it must be remembered that while moist steam under pressure is more effective than streaming steam, it is scarcely necessary to give it the preference, in view of the fact that most known pathogenic bacteria produce no spores and the spores of the few that do develop them are quickly destroyed by the temperature of boiling water, and also that "superheated" steam is less effective than moist steam. When confined steam in pipes is "superheated" after its generation it has about the same germicidal power as hot, dry air at the same temperature. Esmarch found that anthrax spores were killed in streaming steam in four minutes, but were not killed in the same time by superheated steam at a temperature of 114° C. It should also be remembered that dry heat has but little penetrating power, and that even steam requires time to pass through heavy goods. Koch and Wolffhügel found that registering thermometers placed in the interior of folded blankets and packages of various kinds did not show a temperature capable of killing bacteria after three hours' exposure in a dry hot-air oven at 133° C. and over. We have put a piece of ice in the middle of several mattresses and recovered it after exposing the goods to an atmosphere of live steam for ten minutes.

Fractional Sterilization.—Certain nutrient media, such as blood-serum and the transudates of the body cavities, as well as certain fluid foodstuffs, need at times to be sterilized, and yet cannot be subjected to temperatures high enough to kill spores without suffering injury. The property of spores, when placed under suitable conditions, to germinate into the non-spore-bearing form, is here taken advantage of by heating the fluids up to the highest non-injurious point for a certain time on each of several consecutive days, and keeping the fluid at about 20° C. during the intervals. By this means we kill, upon each exposure, all bacteria in vegetative form, and allow during the intervals, for the development of any still remaining in the spore stage, or which have reproduced spores, to change again into the vegetative form. Experience has shown that, with but few exceptions, in the case of blood serum and body transudates, an exposure for six consecutive days at 55° to 70° C. for one hour will completely sterilize the fluids so exposed.

With the usual culture media a temperature of 100° C. for twenty minutes does little or no harm, while one of 120° C. is sometimes deleterious. With heating to 100° C. an exposure on three consecutive days, and to 115° C. on one or two days suffices.

Pasteurization.—It is sometimes undesirable to expose food, such as milk, to a temperature that will destroy spores, because of the deleterious effects of such high temperatures upon food values, and yet a partial destruction of the contained bacteria is necessary. In these cases we heat the foodstuffs for from twenty to forty minutes at 60° C. or from two to five minutes at 70° C. This degree of heat

will kill the bacteria in the vegetative form, but allow the spores to remain alive. These exposures kill about 98 to 99 per cent. of the bacteria in milk. The exposure to this degree of heat alters the chemical composition of the milk but little.

4. Influence of Light.—A large number—perhaps the majority—of bacteria are inhibited in growth by the action of bright daylight, all are by that of direct sunlight, and when the action of the latter is prolonged they lose their power of developing when later placed in the dark.

In order to test the susceptibility of bacteria to light, it is best, according to H. Buchner, to suspend a large number of bacteria in nutrient gelatin or agar and pour the media while still fluid in Petri dishes, upon which has been pasted a strip of black paper on the side upon which the light is to act. The action of heat may be excluded by allowing the ray of light first to pass through a layer of water or alum of several centimetres' thickness. After the plates have been exposed to the light for one-half, one, one and a half, two hours, etc., they are taken into a dark room and allowed to stand at 20° or 35° C. a sufficient length of time to allow of growth, and then examined to see whether there are colonies anywhere except on the spot covered by the paper; when the colonies exposed to the light have been completely destroyed there is lying in a clear sterile field a sharply defined region of the shape of the paper strip crowded with colonies.

Dieudonné, in experiments upon the *Bacillus prodigiosus*, found that direct sunlight in March, July, and August killed these bacilli in one and a half hours; in November in two and a half hours. Diffuse daylight in March and July restrained development after three and a half hours' exposure (in November four and a half hours) and completely destroyed their vitality in from five to six hours. The electric arc light inhibited growth in five hours and destroyed vitality in eight hours. Incandescent light inhibited growth in from seven to eight hours and killed in eleven hours. Similar results have been obtained with *B. coli*, *B. typhosus*, and *B. anthracis*. According to Koch, the tubercle bacillus is killed by the action of direct sunlight in a time varying from a few minutes to several hours, depending upon the thickness of the layer exposed and the season of the year. Diffuse daylight also had the same effect, although a considerably longer time of exposure was required—when placed close to a window, from five to seven days. *B. diphtheriæ* protected by clear, non-colored glass is not materially affected by diffuse daylight or by direct sunlight. Unprotected they are quickly killed by the latter and slowly by the former.

Only the ultraviolet, violet, and blue rays of the spectrum seem to possess bactericidal action; green light has very much less; red and yellow light none at all. The action of light is apparently assisted by the admission of air; anaërobic species, like the tetanus bacillus, and facultative anaërobic species, such as the colon bacillus, are able to

withstand quite well the action of sunlight in the absence of oxygen, the *B. coli* intense direct sunlight for four hours.

According to Richardson and Dieudonné, the mechanism of the action of light may be at last partially explained by the fact that in agar plates exposed to light for a short time (even after ten minutes' exposure to direct sunlight) hydrogen peroxide (H_2O_2) is formed. This is demonstrated by exposing an agar plate half covered with black paper, upon which a weak solution of iodide of starch is poured, and over this again a dilute solution of sulphate of iron; the side exposed to the light turns blue-black. In gases containing no oxygen, hydrogen peroxide is not produced, and the light has no injurious effect. Access of oxygen also explains the effect which light produces on culture media which have been exposed to the action of sunlight, as standing in the sun for a time, when afterward used for inoculation. The bacteria subsequently introduced into such media grow badly—far worse than in fresh culture media which are kept in the shade.

Influence of Radium.—*Radioactive fluids* have a slight inhibiting effect on bacterial growth, but nothing decided enough to be used for therapeutic purposes has been evolved up to the present time.

Influence of X-Rays.—These rays have a slight inhibiting effect on bacteria when they are directly exposed to them.

5. Influence of Electricity on Bacteria.—The majority of the observations heretofore made on this subject would seem to indicate that there is no direct action of the galvanic current on bacteria; but the effect of heat and the electrolytic changes in the culture liquid resulting from the electrolysis may destroy them.

6. Influence of Agitation.—Meltzer has shown that the vitality of bacteria is destroyed by protracted and violent shaking, which causes a disintegration of the cells. Appel found that moderate agitation of the bacteria caused no injury, even when long continued.

7. Influence of Pressure.—Bacteria in fluids which are subjected to great pressure are for a time inhibited in their growth. When oxygen or nitrogen are used the same moderate inhibition occurs.

Influence of Carbonic Acid Under Pressure.—D'Arsonval and Charrin submitted a culture of *Bacillus pyocyaneus* to a pressure of fifty atmospheres under carbonic acid. At the end of four hours cultures could still be obtained, but the bacillus had lost its power of pigment production. A few colonies were developed after six hours' exposure to this pressure, but after twenty-four hours no development occurred. Other bacteria subjected to pressure have exhibited more resistance. We have subjected broth and milk containing typhoid, dysentery, diphtheria, and colon bacilli to the gas under a pressure of seventy-five and one hundred and fifty pounds. Within twenty-four hours 99 per cent. of those in the broth and 98 per cent. of those in the milk were destroyed. Within one week the broth was sterile and within four weeks the milk was sterile. Tubercle bacilli and staphylococci were much more resistant, but little effect being noticed in twenty-four hours. The results were the same whether

the cultures were kept at 10° or 25°. Bottled waters charged with carbonic acid are usually sterile.

8. Life in Absence of Moisture.—For growth, bacteria require much moisture. Want of water affects them in different ways. Upon dried culture media development soon ceases; but in media dried gradually at the room temperature (nutrient agar, gelatin, potato) they live often for a long time, even when there are no spores to account for their longevity A shrunken residue of such cultures in bouillon has often been found, after a year or more, to yield living bacteria. The question as to how long the non-spore-bearing forms are capable of retaining their vitality when dried on a cover-glass or silk threads has been variously answered. We know now that there are many factors which influence the retention of vitality; spores, of course, being more resistant than vegetive forms. The following table of the results obtained by Sirena and Alessi with some non-spore-bearing forms, gives some idea of the extent and effect of such influences. In the experiments silk threads were saturated with bouillon cultures or aqueous suspensions of the bacteria, and some then enclosed in tubes containing sulphuric acid or calcium chloride, while others were left exposed to various outside influences:

Desiccation	With sulphuric acid, killed at end of	With calcium chloride, killed at end of	In incubator at 37°, killed at end of	In dry room in shade, killed at end of	In moist room, killed at end of
Cholera spirilla	1 day	1 day	1 day	1 day	12 days
B. of fowl cholera	2 days	1 day	1 day	5 days	59 days
B. typhosus	41 days	1 day	18 days	64 days	68 days
B. mallei	35 days	44 days	31 days	.....	.....
Diplococ. pneumoniæ	114 days	31 days	131 days	164 days	192 days

The results of all investigators, however, would seem to indicate that the greatest possible care must be exercised in desiccation experiments to come to any positive conclusions; but recently most astonishing results have been obtained with regard to many species usually supposed to be particularly sensitive to desiccation, showing that under certain conditions they may retain their vitality in a dry state for a very long time. Thus, Koch found that cholera spirilla lived only a few hours when dry; Kitasato determined their life duration at fourteen days at most; while various French observers have found that they may, under favorable conditions, live 150 to 200 days. The varying results sometimes reported by different observers in such experiments may be explained by the fact that the conditions under which they were made were different, depending upon the desiccator used, the medium upon which the cultures were grown, and the use of silk threads or cover-glasses. In all these experiments, of course, it should be previously determined that in spore-bearing species there are no spores present. Even when a dried culture lives for a long time the majority of the organisms die in a few hours after drying. We have found 1,500,000 colon bacilli to be reduced to 100,000 after three

hours' drying. When protected by a covering of mucus, as in expectoration, they live much longer than when unprotected.

Duration of Life in Pure Water. — When bacteria which require much organic food for their development (and these include most of the pathogenic species) are placed in distilled water they soon die— that is, within a few days; even in sterilized well water or surface water their life duration does not usually exceed eight to fourteen days, and they rarely multiply. Instances, however, of much more extended life under certain conditions are recorded.

9. Tactic Effect of Chemicals.—*Chemotaxis.*—The deleterious effect of chemicals, especially those used as germicides, will be considered in a separate chapter.

Some chemical substances exert a peculiar attraction for bacteria, known as *positive chemotaxis*, while others repel them—*negative chemotaxis*. Moreover, all varieties are not affected alike, for the same substances may exert on some bacteria an attraction and on others a repulsion. Oxygen, for example, attracts aërobic and repels anaërobic bacteria, and for each variety there is a definite proportion of oxygen, which most strongly attracts. The chemotactic properties of substances are tested by pushing the open end of a fine capillary tube, filled with the substance to be tested, into the edge of a drop of culture fluid containing bacteria and examining the hanging drop under the microscope. We are able thus to watch the action of the bacteria and note whether they crowd about the tube opening or are repelled from it. Among substances showing positive chemotaxis for nearly all bacteria are peptone and urea, while among those showing negative chemotaxis are alcohol and many of the metallic salts. Such experiments are of course rough. The diffusion of the substances from the tube into the surrounding medium must play an extremely active rôle in the final result.

CHAPTER V.

THE MATERIALS AND METHODS USED IN THE CULTIVATION OF BACTERIA.

THE methods employed for the artificial cultivation of bacteria are of fundamental importance in bacteriology. The study of the characteristics of any bacterium requires that it be examined growing apart from all others in pure cultures. In order to separate one species from others and to study its morphologic, biochemic, and cultural characteristics we have to prepare a number of sterile solid and liquid media and employ them in various technical ways. In the first place, however, we have to take the greatest precautions to insure that the materials that we make use of for the growth of bacteria, the flasks and tubes that hold these materials, and the instruments with which we transfer the bacteria are sterile.

Cleansing and Sterilization of Apparatus.—In bacteriologic work sterilization is practically always done by means of dry and moist heat, for no antiseptic substances can be allowed to remain in any of the media used for the growth of bacteria or on any of the apparatus which would come in contact with them, as such substances would inhibit the growth of the bacteria which we desire to study.

The platinum wires and loops (Fig. 54) used in transferring bacteria are sterilized by holding them for a moment until red-hot in a gas or alcohol flame. They should not be used until time enough has elapsed for them to cool sufficiently not to injure the bacteria touched by them. Knives, instruments, etc., are, after thorough cleansing, placed in boiling 1 per cent. washing soda solution for three to five minutes. Hypodermic needles are sterilized by boiling in soda solution, or, when this is impossible, they are first frequently rinsed with boiling or with very hot water, and then filled with a 5 per cent. carbolic acid solution for at least thirty minutes and then rinsed again with sterile water. New tubes and flasks sometimes require to be washed in a solution of dilute hydrochloric acid, so as to remove any free alkali which may be present. They are finally thoroughly rinsed in pure water. Old tubes, flasks, and other glassware are boiled for about thirty minutes in a 5 per cent. solution of washing soda in soapsuds, and then thoroughly rinsed off with water until perfectly clean. If necessary, any dirt clinging to the insides of the flasks and tubes can be removed by bristle brushes or suitable swabs. After the tubes and flasks have been thoroughly cleaned they are plugged loosely with ordinary cotton-batting, or, if that is not at hand, the more expensive absorbent cotton. The tubes and flasks with their cotton plugs and all other glassware

are sterilized by dry heat at 150° C. for one hour in the dry-heat sterilizer (Fig. 31).

Preparation of Culture Media.—Before we can get a suitable growth of any special variety of bacteria, we must have the substances necessary for growth present in the proper proportion and concentration. Certain species of bacteria require special foodstuffs, so that for each kind the proper food must be found through experimentation. The most commonly used media have as their basis the watery extract of meat and peptone. The addition to this by Koch of gelatin gave us a transparent solid medium which had, however, the objection of melting below the temperature required for the growth of many pathogenic bacteria. Another substance, of vegetable origin (agar), was found, which melted just below the boiling point of water. This has been substituted for gelatin whenever we desire to grow bacteria at temperatures above 20° C. or desire other characteristics of the agar media.

FIG. 31

Dry-heat sterilizer.

Preparation of Meat Infusion and Simple Bouillon.—One pound (500 grams) of finely chopped, fresh, lean meat is macerated in 1000 c.c. of water and put in an ice-chest for from eighteen to twenty-four hours; or it may be warmed at a temperature not exceeding 60° C. for one hour. Any fat present is skimmed off. The last traces can be removed by stroking the surface with filter-paper. The infusion is now strained through a fine cheese-cloth into a flask, and the remaining meat placed in a cloth and squeezed by hand or in a press. The resulting fluid contains the soluble albumin, the soluble carbohydrates, the soluble salts, extractives, and coloring matter of the meat. This meat extract is then exposed to live steam, either without pressure in the Arnold steam sterilizer (Fig. 32) for thirty minutes, or, if the changes produced by a temperature of 110° to 115° C. are not objectionable, in the autoclave at a pressure of one atmosphere for fifteen minutes, or boiled over a free flame for ten minutes. During this process all the albumins are coagulated. While still hot the fluid is filtered through filter-paper or through absorbent cotton, and the reaction is tested and sufficient normal hydrochloric acid solution or sodium hydroxide added to give it the desired reaction, which is for most bacteria slightly alkaline to litmus (1 per cent. acid to phenolpthalein, the standard indicator).[1] If in the boiling there has been any evaporation, sufficient water is added to bring the fluid up to its original bulk. If the fluid is clear it is put into flasks and tubes and sterilized; if not clear, the white of one or two eggs beaten up in water (50 c.c. to an egg) is added to the fluid after cooling it down to about 55° C. After thoroughly mixing with the eggs, the bouillon is boiled

[1] The method of titration is given later on p. 67.

briskly for a few minutes, its reaction adjusted, and then again filtered and distributed in flasks and put in the Arnold sterilizer for one hour on each of three consecutive days, or in the autoclave for twenty minutes for sterilization.[1] Instead of meat 2 to 4 grams of Liebig's or some other meat extract may be added to each litre of water. It is best to dissolve the extract in a small amount of cold water and filter through a cold wet filter-paper to remove the excess of fat which occurs frequently in certain meat extracts. For some purposes the extract is as good as the fresh meat, but for others it is inferior. This simple bouillon contains very little albuminous matter, and consists chiefly of the soluble salts of the muscle, certain extractives, and any slight traces of soluble proteid not coagulated by heat. It is not, therefore, a suitable medium for most bacteria.

FIG. 32

Arnold steam sterilizer.

We use this or the infusion as a basis for the following more useful media:

Nutrient Bouillon Media.—These consist of meat infusion plus certain nutrient substances.

(*a*) *Peptone or Nutrient Bouillon.*—This has the following composition: meat infusion, 1000 c.c.; sodium chloride, 5 grams; peptone (Witte), 10 grams. Warm moderately and stir until the ingredients are dissolved, then boil for thirty minutes in the Arnold sterilizer or the autoclave and treat as in making simple bouillon. For the careful study of bacteria the exact reaction of the media should be carefully determined. For this purpose standard solutions are used with phenolphthalein or litmus as an indicator. This subject will be taken up in detail later in this chapter. For water bacteria sodium chloride is omitted and the reaction is made + 1 per cent.

(*b*) *Sugar-free Nutrient Bouillon.*—A quantity of a culture of bacillus coli or of bacillus lactis aërogenes is added to the meat extract and incubated at 37° for twenty-four hours. The acidity is neutralized, peptone and salt added, and treated as described under bouillon.

(*c*) *Sugar Nutrient Bouillon.*—To the sterile peptone broth from which, before its completion, the fermentable sugars have been removed 1 to 2 per cent. of glucose, lactose, saccharose, or other sugar is added. No more boiling than necessary to sterilize should be used after the addition of the sugars, since they become altered by heat. Temper-

[1] After heating the reaction may become more acid by the releasing of free H ions from the phosphates present.

atures higher than 100° C. should never be employed. These media are used to determine the effect of bacteria upon the different sugars.

(*d*) *Glycerin-peptone Nutrient Bouillon.*—After filtration, 3 to 5 per cent. of glycerin is added to the peptone bouillon and the whole again sterilized. This medium is used especially for the growth of the tubercle bacilli.

(*e*) *Mannite-peptone Bouillon.*—This is prepared by adding 1 per cent. mannite to the peptone bouillon. It is used especially in differentiating between the varieties of dysentery bacilli, some fermenting mannite and others not. In careful work the bouillon must be rendered sugar free.

Bouillon for Production of Diphtheria Toxin.—This is now prepared as follows, in the Research Laboratory of the Health Department:

The clean muscle of young veal, preferably "Bob veal," is chopped up and tap water added in the usual manner. This is allowed to ferment over night at room temperature, about 24° C. It is then digested for two hours at 55° C. The infusion is now boiled for thirty minutes. The boiled fluid is strained from the meat and receives 2 per cent. peptone and ½ per cent. salt. The broth is then titrated at room temperature using phenolpthaline for an indicator. The first faint pink color is used to indicate the end of the reaction. Sufficient normal sodium hydrate is added to bring the acidity down to 1.2 per cent. normal acid. The broth is boiled again for twenty minutes and filtered clear.

Gelatin Media.—These are simply the various bouillon and peptone media to which gelatin is added as follows: To the nutrient bouillon already prepared as described add 10 per cent. of sheet gelatin and neutralize. Add the whites of two eggs for each litre, and boil for a few minutes. Filter, place in tubes or flasks, and sterilize. After sterilization the gelatin should be placed at once in a cool place. This procedure prevents a further lowering of the original melting point. Instead of adding gelatin to bouillon already prepared, it may be added to the meat infusion at the same time the peptone and salt were added in preparing nutrient bouillon as just described. Different preparations of gelatin differ greatly as to their melting point. Boiling lowers the melting point, so that heat should not be applied any longer than necessary. The melting point of different samples of nutrient gelatin varies between 20° to 27° C. The "gold-label" gelatin is employed.

Agar Media.—These are the various bouillon and peptone media to which 1 to 2 per cent. of agar-agar are added. When sugars are needed, in order to lessen the effect of heat on them, simple nutrient agar is first prepared and then the sugar added. Nutrient agar is prepared by adding to stock bouillon 1 to 2 per cent., as desired, of thread agar, melting it by placing over a free flame or in the autoclave or steam sterilizer. When the agar is brought into solution over a free flame there may be considerable loss of fluid by evaporation. This should be compensated for by adding additional water

before boiling. Agar may be added directly to the meat infusion along with the peptone and salt. Indeed, this is an advantage, as agar-agar is very difficult to bring into solution, and is not injured in the least by prolonged boiling. The agar may be added to water alone in double the amount finally desired. To this is added an equal quantity of nutrient broth, which is also double its usual strength. Nutrient agar begins to thicken at a fairly high temperature, and should be filtered as hot as possible. When small amounts are made it is well to place the filter and receiving flask in the sterilizer while filtering.

Glycerin agar is simply nutrient agar plus 3 to 6 per cent. of glycerin. It is added to the hot nutrient agar just previous to putting it in the flasks.

The following special media are also used in the cultivation of bacteria:

Peptone Solution (Dunham's).—This is a simple 1 to 2 per cent. solution of peptone in tap or distilled water to which 0.5 per cent. of sodium chloride is added. The peptone and sodium chloride are dissolved by heating. The fluid is filtered, placed in tubes, and sterilized. A reaction slightly alkaline to litmus is suitable for most purposes. It can be altered or standardized if desired.

Sugar-peptone Solution, etc.—The various sugars and mannite, inulin, glycerin, etc., are added to the peptone solution just as previously described for bouillon.

Milk.—This fluid is a good culture medium for most pathogenic bacteria. It should be obtained as fresh as possible, so that but little bacterial change has occurred. It is first put in the ice-chest for twelve hours to allow the cream to rise. The milk is then siphoned off from below the cream into a flask and its reaction tested. After correction it is put in tubes or flasks and sterilized. If acid to phenolphthalein, normal sodium hydrate should be added to make it —1 per cent.

Litmus Media.—When it is desirable to determine whether bacteria produce in their growth acid or alkali from one or more of the constituents of the media, litmus is frequently added. To prepare the litmus solution take Merck's purified litmus, powder finely, and make a 5 per cent. solution in distilled water. Steam this in Arnold's sterilizer for two hours, shaking frequently. Filter and then boil for thirty minutes on two successive days. The litmus solution is added to the neutral media in sufficient quantity to give the desired depth of color. The less heating that is done after mixing the better the results.

Petrusky's Litmus Whey (as modified by Durham).—Fresh milk is slightly warmed and clotted by means of essence of rennet. The whey is strained off and the clot is hung up to drain in a piece of muslin. The whey, which is somewhat turbid, is then cautiously neutralized with 4 per cent. citric acid solution, neutral litmus being used as an indicator. When it gives a good neutral violet color with the litmus it is heated at 100° C. for one hour; thereby nearly the whole proteid is coagulated.

It is thus filtered clear, and neutral litmus is added to a convenient color for observation.

Neutral Red.—This dye is added to the peptone and bouillon-sugar media to the amount of 1 to 5 per cent. of a concentrated solution. Its reduction by the growth of bacteria is a valuable point in differentiation in certain cases.

Nitrate Bouillon.—Dissolve 10 grams of peptone in 1 litre of spring or tap water and add 0.02 gram of potassium nitrate (which is free of nitrites). This is placed in test-tubes and sterilized.

Potatoes.—Potatoes are used for some special purposes. The potatoes may, after thorough scrubbing and removal of "eyes," be soaked in bichloride of mercury (1:1000) for twenty minutes, placed in running water twenty-four bours to prevent darkening, and then sterilized on three consecutive days for one-half hour in the steam sterilizer. To use they are cut in thick slices and put in deep Petri dishes. When desired the potatoes are first cut into proper sizes for tubes, and then soaked for twelve hours in one per cent. sodium carbonate solution to remove the acidity.

Bile.—Fresh bile of cattle is sterilized and used without additions or to it is added 1 per cent. of peptone or again 10 per cent. of peptone and 10 per cent. of glycerin. The bile inhibits the coagulation of blood and also the development of many varieties of bacteria. The bacilli of the colon-typhoid growth are, however, little affected. This medium is used especially for obtaining the typhoid bacillus from the blood and from water, and the colon bacillus from polluted water.

Blood Media.—(*a*) *Fresh Blood Media.*—These are made by streaking sterile defibrinated or fresh human, rabbit, or other blood over nutrient agar contained in tubes or dishes. Sometimes fresh blood is added to fluid nutrient agar at 40° C. or to bouillon and a mixture thus obtained. Media made with fresh blood contains not only the hæmoglobin, but also intact red blood cells. Blood media are used for the growth of the influenza bacillus, for pneumococci and other bacteria, and for the observation of the production of hemolysis by the growth of certain bacteria.

(*b*) *Heated Blood Media.*—The clot containing the red cells, after the separation of the serum, is broken up and added to the bouillon and heated to 80° to 90° C. This makes a muddy fluid which is fitted only for the development of bacteria where no exact observation of their growth characteristics is required.

Blood-serum Media. Ascitic or Pleuritic Fluid.—Blood serum may be sterilized by fractional sterilization and remain fluid, or it may be rendered solid by the degree of heat used in sterilizing. The blood may be obtained from an ox, horse, sheep, dog, or rabbit and collected into jars, flasks, or tubes, where it is allowed to stand until it clots. When the serum is to be used in a fluid state the blood should be drawn in an aseptic manner into a flask from a vein by means of a sterile cannula and rubber tube. When the serum is to be solidified, less care is necessary. It is here sufficient to catch the blood from the cut artery or vein into sterile jars or tubes. To facilitate clotting it is well to

have in the jar or tube something upon which the clot may contract, such as nickel-plated wire or broken glass.

Loeffler's Blood Serum.—Three parts of calf's or sheep's blood serum is mixed with one part of neutral peptone bouillon containing 1 per cent. of glucose. The serum mixture is run into tubes, which are plugged and then placed in a slanting position in the serum coagulator.

Serum may be solidified and still remain translucent at a temperature of 76° C., but when heated to a higher degree a more definite coagulation takes place, and the medium becomes opaque. Care must be taken in coagulating blood serum at the higher temperature to run the temperature up slowly, and not to heat above 95° C. until the serum has firmly coagulated; for, unless these precautions are taken, ebullition is likely to occur, which will lead to the formation of bubbles and an unevenness of the surface upon which growth is to be obtained and studied. Serum may be solidified at the temperature mentioned in an incubator, water-oven, or even in an Arnold sterilizer with the top covered by a cloth instead of the usual lid, and when coagulated firmly (90° C.) the tubes and their contents may, on the following day, be sterilized in streaming steam at 100° C. without danger of the subsequent formation of bubbles. Koch's serum coagulator (Fig. 33) is, however, the most convenient apparatus. A modification of this which we made is very useful. The water holder is 10 inches high and into it are built three boxes having the proper slant, and opening in front. Each compartment has a cover. The serum-holding tubes are inserted in the boxes. In this way the warm water is above as well as below, so that the heating is uniform. Some bacteriologists prepare the tubes of solidified serum in the autoclave, gradually increasing the temperature to 110° C. This is a very rapid and convenient method. It has seemed to us, however, that the high temperature injured the medium somewhat.

FIG. 33

Blood-serum coagulator.

Alkaline Blood Serum.—To each 100 c.c. of blood serum add 1 to 1.5 c.c. of a 10 per cent. solution of sodium hydrate. Treat as Loeffler's serum. This will give a solid, clear medium consisting chiefly of alkali albuminate.

Serum-bouillon Media (Marmorek's Media):

1. Human serum, 2 parts; nutrient bouillon, 1 part.
2. Ascitic or pleuritic fluid, 1 part; nutrient bouillon, 2 parts.
3. Horse serum, 1 to 2 parts; nutrient bouillon, 1 to 2 parts.

These media were first used extensively by Marmorek in cultivating streptococci. The ascitic fluid bouillon has been found by Williams to be of great use in enriching cultures of diphtheria bacilli. It is

also one of the best media for the growth of pneumococci, streptococci, and many other pathogenic bacteria.

Serum-water Media (Hiss' Serum Media).—When diluted with 2 to 10 parts of water, many sera can be steamed without coagulating.

1. Ox serum, 1 part; distilled water, 2 parts; normal sodium hydrate, 0.1 per cent.

2. The same, with inulin 1 per cent. substituted for the sodium hydrate.

For the sterilization of undiluted fluid serum and of ascitic and pleuritic fluids, it is requisite that they be exposed to a temperature of from 62° to 66° C. for one hour on each of six consecutive days. The best apparatus for obtaining and maintaining this temperature (about 65° C.) is a small and well-regulated incubator or chamber surrounded by a water space, into which the tubes and flasks containing serum are to be put each day, and in which they are to be left for the prescribed time after having been warmed to the desired temperature.

Serum may be preserved by placing it in flasks which, after the addition of 5 per cent. of chloroform, are sealed. When it is to be used it is poured into sterilized culture (test) tubes and sterilized by exactly the same methods as are employed in sterilizing fresh serum. The chloroform, being volatile, tends to disappear at ordinary temperatures, but is quickly and surely driven off at the temperatures used in sterilizing.

Serum may be efficiently sterilized, when great care is used, by passing it through a well-tested Pasteur filter, under pressure. When so treated the fluid is very clear and light colored. The first few cubic centimetres are deficient in blood proteids because of adhesion to the filter.

Important media used for special varieties of bacteria will be noted in the chapters devoted to these bacteria.

Reaction of Culture Media.—The reaction of media is a matter of the greatest importance, since slight variations will often aid or inhibit the growth of bacteria and also produce marked differences in the microscopic and macroscopic characters of a growth.

Formerly it was customary to use litmus as the indicator in neutralizing media, adding normal soda solution or hydrochloric acid solution until the red litmus turned blue, or the blue litmus just a tinge less blue. This was considered the neutral point. This method is still a satisfactory one for those who are only going to cultivate the common pathogenic bacteria for diagnostic purposes or for the routine development of toxin. Most parasitic bacteria which grow at all on artificial culture media develop best in them when they have a neutral or slightly alkaline reaction to litmus. If a certain alkalinity is desired a definite number of cubic centimetres of normal soda solution can be added for each litre of neutral media; if an acidity is desired, normal hydrochloric acid solution is added.

Many bacteriologists consider that litmus is not delicate enough to be entirely satisfactory, especially when experiments are to be

reported or exactly repeated. This objection is made chiefly by those investigating water bacteria who are watching cultural and biochemic characteristics in simple peptone-beef media. For these purposes phenolphthalein has been generally selected. It is of great importance to remember that different indicators not only differ in delicacy, but that they react differently to different substances. A medium which is slightly alkaline to litmus is usually slightly acid to phenolphthalein, showing that there are present in such media substances possessing an acid character which litmus does not detect. These substances are weak organic acids and organic compounds, theoretically amphoteric, but in which an acid character predominates. Thus, a litre of bouillon becomes, on the addition of 1 per cent. of peptone, more alkaline to litmus, but decidedly more acid to phenolphthalein; 100 c.c. of water with 1 per cent. of peptone is acid to phenolphthalein to such an extent that about 3.5 c.c. of decinormal NaOH is required to neutralize it. To litmus it is alkaline and requires 3.4 c.c. of decinormal HCl. Two per cent. of peptone doubles the difference. The same figures hold approximately true for peptone broth. We should find by growing the bacteria just what reaction we want for any variety, and then test the fluid with phenolphthalein or litmus as the indicator. With precisely similar ingredients we can then exactly reproduce at any time in the future the same reaction, but with different materials one would again have to study the reaction.

Titration of Culture Media.—We must have accurately standardized normal and decinormal solutions of sodium hydrate and hydrochloric acid; also a 0.5 per cent. solution of phenolphthalein in 30 per cent. alcohol and a neutral 1 per cent. solution of Merck's litmus.

Care should be taken to prevent the absorption of carbon dioxide by the soda solution, by arranging that all air which comes in contact with the latter, either in the stock bottle or in the burette, shall first pass through a strong solution of sodium or borium hydrate. The arrangement of the apparatus is described in any work on chemical analysis. The medium is brought to the desired volume with water and boiled four minutes to expel the carbon dioxide. Media are commonly warm or hot when measured, hence it must be remembered that true volumes cannot be thus obtained; for instance, a litre measured at, say, 80° C. would be only 973 c.c. if measured at 20° C., the temperature at which litre flasks are calibrated. Since many media cannot be cooled to 20° C. because of solidification, as in the case of agar or gelatin, it is a better plan when accuracy is important to determine measures of volume by weight. For this, place a clean, dry saucepan, in which the medium is to be prepared, upon one side of a trip scale, and counterbalance its weight exactly. The weight of a litre of bouillon, gelatin, or agar having been determined once for all, the necessary weights added to the weight of the pan will give the amount which the pan and its contents must balance when the volume is exactly one litre. A portion of the medium brought to the exact volume is then taken and cooled to room temperature

(20° C.), or to a point a few degrees above solidification, and 10 c.c. withdrawn, placed in a small beaker, 50 c.c. of distilled water and 1 c.c. of the phenolphthalein solution added. If the medium is acid the $\frac{N}{10}$ NaOH solution is then run in cautiously until a pale but decided pink color is obtained. The number of cubic centimetres of the solution used, multiplied by ten, will give the number of cubic centimetres of normal sodium hydrate per litre necessary to effect complete neutralization. The question as to what is the best reaction of media for general work is not an easy one to settle, and one on which bacteriologists differ. What is the proper reaction for one variety of bacteria is often far from the best for some other variety. Reactions are now commonly expressed by plus or minus signs, the former representing an acid and the latter an alkaline condition, the number following the sign representing the percentage of normal acid or alkali present in the medium. Thus, +1.5 would indicate that the medium contained 1.5 parts per 100 or 1.5 per cent. of free nor-

FIG. 34

Erlenmeyer flask.

FIG. 35

Long-necked flask.

FIG. 36

Pasteur flask.

mal acid, while —1.5 would indicate that the medium contained an equivalent quantity of free alkali. The committee of the American Public Health Association in 1898 adopted for nutrient bouillon or agar a reaction of +1.5 as the best for general work in water examinations. In 1905 this was changed to +1.0 per cent. A medium whose reaction is +0.5 per cent. acid to phenolphthalein is still better adapted for many bacteria. It cannot be too strongly impressed upon the reader that whatever the reaction, its measure should be stated in all descriptions of cultural characters. The litmus solution is added in the same way as that of phenolphthalein.

Storage of Media.—The nutrient media are stored in glass flasks (Figs. 34 and 35). From these, as needed, glass tubes are filled. When small amounts of media are taken frequently from flasks, Pasteur's flasks (Fig. 36) are of great convenience. They consist of a flask with a ground-glass neck, over which fits a cap. This cap may or may not terminate, as desired, in a narrow tube, which is plugged with cotton. The cap keeps the edges of the flask free from bacteria and prevents the cotton from sticking. A tumbler or a simple cap of paper over the

neck answers much the same purpose. Stock media, unless protected from drying by sealing, should be kept in a cool moist place until needed.

Preparation and Filling of Tubes.—The cheaper grades of test-tubes should be avoided. They are thin and break easily, and also frequently frost on heating, from the separation of silicic acid. The tubes of the better class can be used after rinsing with hot water; they should have no lip. Cheap tubes are very alkaline and must first be soaked in dilute hydrochloric acid. The sterilization of glassware has already been spoken of (p. 59).

The sterile tubes and flasks are filled with the media, when small quantities are used, by means of a sterile glass funnel. The main precaution to be observed is not to let the media soil the neck of the tubes and flasks, as this would cause the fibres of the cotton plugs to adhere to the sides of the tubes when the media dried, and make it difficult to remove the plugs wholly when we wished to inoculate the contents of the tubes.

The tubes and flasks, plugged with sterile cotton and containing media, are sterilized by fractional sterilization at 100° C. for one-half hour on three consecutive days; or they may be sterilized by steam under pressure (in autoclave for fifteen minutes) on two consecutive days. A portion of the tubes containing nutrient agar are laid in a slanted position before cooling, after the final sterilization, so that a larger surface may be obtained.

THE CULTIVATION OF BACTERIA.

Bacteria can seldom be identified by their microscopic and staining characteristics alone. By these methods only their individual forms, arrangement, and motility or lack of motility can be studied. To go beyond this we have to grow the microörganism in pure culture on the various culture media and perhaps also in animals. It is necessary, as well, to have the proper conditions as to temperature, moisture, access of oxygen, etc.

When we make cultures from any material, we are very apt to find that instead of one variety of bacteria only there are a number present. If such material is placed in *fluid* media contained in test-tubes, we find that the different varieties all grow together and become hopelessly mixed. When, on the other hand, the bacteria are scattered over or through *solid* media they develop about the spot where they happen to light, forming small *colonies* each composed of a single variety of organism. If different varieties, however, are placed too near together, they overgrow one another; it is thus advisable to have a greater surface of nutrient material than is given on the slanted surface of nutrient agar or blood-serum contained in test-tubes. This need is met by pouring the media while warm on flat, cool, glass plates or into shallow dishes. From the isolated colonies thus formed new growths may be obtained of a single variety, and thus we have a *pure culture* (see p. 75).

Technique of Making Plate Cultures.—In making plate cultures two methods are carried out. In the first the material with its contained bacteria is scattered throughout the fluid before it hardens; in the second it is streaked over the surface of the medium after that has solidified. Nutrient agar and nutrient gelatin, the two substances used for plate cultures, differ in two essential points, which cause some difference in their uses. Nutrient 1 per cent. agar melts, near the boiling point and begins to thicken at about 36° C. It is not liquefied by bacterial ferments. Nutrient 10 per cent. gelatin melts, according to the variety used, at the low temperature of about 23° to 27° C., and solidifies at a point slightly below that. It is liquefied by many bacterial ferments. When we wish to inoculate fluid nutrient agar for plate cultures we have to take great care that in cooling it to a point which will not injure the bacteria, about 41° C., we do not allow it to cool too much and thus solidify and prevent our pouring it into the plates. The correct way to proceed when a number of tubes are to be inoculated, to place them while still hot in a basin of water which has been heated to about 45° C. Then when the temperature of the agar in the tubes as shown by a thermometer placed in one of them, has fallen to 42° C., the water, milk, feces, bacterial culture, or other substance to be tested is added to the other tubes or placed in the dishes in whatever quantity is thought to be proper up to 1 c.c. A greater quantity of fluid would dilute and cool the nutrient agar too much. After inoculation, the contents of the tubes are thoroughly shaken and poured out quickly into round, flat-bottomed, glass Petri dishes (Fig. 37), the covers of which are raised on one side for the required time only. Instead of placing the fluid containing the bacteria in the tube it is often placed directly in the Petri dish. In this case no bacteria are left in the medium sticking to the tube from which it is poured, and hence organisms lost. The melted nutrient gelatin or agar is then poured in the dish, and by gently tipping the fluids are mixed. It is somewhat more difficult to scatter the bacteria evenly when they are mixed with the media in plates rather than in tubes so that there is little to choose in point of accuracy between the two methods. The bacteria are now scattered throughout the fluid, and as it quickly solidifies they are fixed wherever they happen to be, and thus, as each individual multiplies, clusters are formed about it at the spot where it was fixed at the moment of solidification. The number of colonies of bacteria thus indicate to us roughly the number of living bacteria in the quantity of fluid added to the liquid gelatin (Fig. 38) or agar. Groups or chains of bacteria which in spite of shaking remain attached produce single colonies. Bacteria which do not grow on the media or at the temperatures employed produce of course no colonies. Nutrient gelatin is used exactly as agar, except that as the average product does not congeal until cooled below 22° C. we have no fear of its cooling too rapidly.

FIG. 37

Petri dish.

In order not only to count the number of colonies and to obtain a characteristic growth, but also to prevent the inhibition of the growth of some and the fusing of others, it is desirable not to inoculate the nutrient agar or gelatin to be poured in one plate with too large a number of bacteria. We therefore use the following dilution methods in making culture plates of suspected material.

Dilution Methods.—As it is impossible to know the number of bacteria in any suspected fluid, it is usual to make a set of from two to four different plates, to each of which a different amount of material is added, so that some one of the series may have the required number of colonies. The dilutions are made in bouillon or sterile distilled water. In the

FIG. 38

Photograph of a large number of colonies developing in a layer of gelatin contained in a Petri dish. Some colonies are only pinpoint in size; some as large as the end of a pencil. The colonies here appear in their actual size.

first tube we place an amount which we believe will surely contain sufficient and probably too many bacteria. To the second tube we add 10 per cent. of the amount added to the first, and to the third 10 per cent. of the second, and to the fourth 10 per cent. of the third. Thus, if the first contained 60,000 bacteria the second would have 6000 (Fig. 38), the third 600, and the fourth 60 (Fig. 39). If, however, the first contained but sixty, the second would have about 6, and the remaining two would probably contain none at all. When there are many colonies present the dishes are covered by a glass plate (Fig. 40), ruled in larger and smaller squares, Wolffhügel's apparatus. With the eye or when necessary aided by a hand lens the colonies in a certain number of squares are counted and then the number for the whole contents estimated. It is very important to remember that when more than 200 or 300 bacteria start to develop in the agar or gelatin contained in a plate some develop colonies which fuse together, while others are inhibited before they develop visible colonies. Thus if sixty thousand

separated bacteria were placed in the agar of one dish they would probably not produce over ten thousand colonies, while one-tenth as much would produce about three thousand and one one-hundredth as much would produce about five hundred. Unless this effect of overcrowding is taken into account gross inaccuracies will occur in estimating the number of bacteria present in the material from which the plates were made. If possible, dilutions should be made so that plates will contain between forty and four hundred colonies. It is often advisable to examine the material to be tested in hanging drop and stained spreads under the microscope in order to determine roughly the number of bacteria present and so decide what dilutions to make.

When the material to be tested is crowded with bacteria it is often

FIG. 39

Well-distributed colonies in agar in portion of Petri dish.

FIG. 40

Wolffhügel's apparatus for counting colonies.

best to make an emulsion of a portion of it, and use this rather than the original substance for making the dilutions to be used. Measured quantities of the diluted material can be transferred most accurately through a sterilized, long, glass pipette graduated in one one-hundredth cubic centimetres, or, more roughly, by a platinum loop of known size.

Streaked Surface Plate Cultures.—About 8 c.c. of agar-agar are poured into a Petri dish and allowed to harden. The substance to be tested bacteriologically, or a dilution of it, is then drawn lightly across the surface of the medium in a series of parallel streaks by means of a platinum loop. Each successive streak is made with the same needle or loop without replenishing the material to be tested. Each streak will therefore leave less deposit of bacteria and fewer colonies will develop. While in the former method (poured plate) most of the bacteria developed under the surface, here all develop upon it. This is an advantage, as many forms of bacteria develop more characteristically on the surface than in the midst of the media, and it is easier to remove them free from other bacteria with the platinum needle. Instead of streaking the material by means of the platinum wire over the agar, a loopful may be deposited on the agar and then smeared over its surface by a sterile swab or a glass rod bent

so that the last two inches strokes the plate horizontally. The old method of using glass plates upon a cooling stage (Koch's method) has now been practically given up for the more convenient one of Petri dishes. In warm weather the dishes may be cooled before using, so as to harden quickly the agar or gelatin that is poured into them.

An old method, which is still sometimes used to find the number

FIG. 41

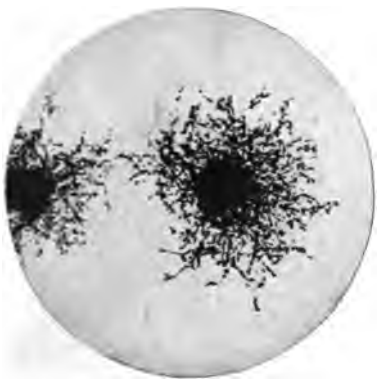


FIG. 42

FIG. 41.—Irregular fringed colony (B. malignant œdema). (From Kolle and Wasserman.)
FIG. 42.—Colonies of typhoid and colon bacilli in rather soft gelatin.

of living bacteria, is, instead of pouring out the media which has been inoculated, to congeal it on the sides of the test-tubes. This is best done by laying the tube flat on its side on a cake of ice and rotating it. Tubes come especially formed for this by having a slight neck, which prevents the media running up to the plugged end of the tube. This method (Esmarch's) is used only when the Petri dishes are not obtainable or cannot easily be transported.

FIG. 43

FIG. 44

FIG. 43.—Colony of colon bacilli grown in soft gelatin.
FIG. 44.—One large irregular colony of colon and two smaller colonies of typhoid bacilli in soft gelatin. (Figs. 42–44 from photographs by Dunham.)

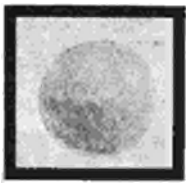
Study of Colonies in Plate Cultures in Nutrient Agar.—The plates should be studied after twelve to forty-eight hours' growth at blood temperature and after two to five days at 70° F. (21° C.). The special time allowed varies according to the rapidity of the growth of the varieties developing; thus, bacteria, such as the streptococci and influenza

bacilli, reach the characteristic development of their colonies in from ten to sixteen hours, while others continue to spread for several days. If we wait too long where numerous varieties of bacteria are growing the colonies of heavier growth may cover up the finer and more delicate

FIG. 45 FIG. 46

FIG. 45.—Moist raised colonies with no visible structure, looking like a drop of water.
FIG. 46.—Deep colonies, usually either light brown, gray, or yellow in color, opaque, with little marking. (Figs. 45–52 from Lehman and Neumann.)

FIG. 47 FIG. 48 FIG. 49



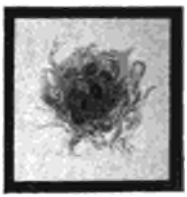


FIG. 47.—The colony very finely granular.
FIG. 48.—Colonies opaque in centre with lighter borders. The margin is coarsely granular, or has twisted threads.
FIG. 49.—Colony in gelatin. The centre is coarsely granular in partly fluid gelatin. The borders are formed of wavy bands of threads.

FIG. 50 FIG. 51 FIG. 52

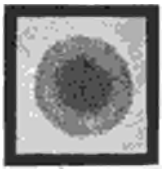


FIG. 50.—Colonies circular in form, composed of radiating threads.
FIG. 51.—Colonies with opaque centres, with a thin border fringe.
FIG. 52.—Colony showing a network of threads which is thicker in centre.

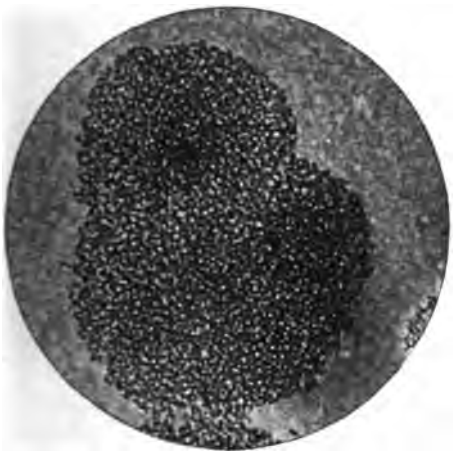
ones. As a rule, the younger colonies are more characteristic, except where the development of pigment is sought.

The colonies are first examined with the naked eye (Fig. 39), then with magnification of about 60 diameters (Figs. 41 to 52), and then,

if necessary, at from 400 to 500 diameters (Fig. 53). We note everything we can about them, such as their size, surface elevation, form, internal structure, edges, and optical characters; if grown in gelatin, whether they have or have not caused liquefaction. The accompanying schematic representations from Lehman and Neumann (Figs. 45 to 52) illustrate some of these points.

At the higher magnification we begin to detect the individual bacteria (Fig. 53). After studying the colonies we remove a few of the bacteria from one or more of them by touching each with the

FIG. 53



Two surfaces colonies of diphtheria bacilli upon agar. × 500 diameters.

FIG. 54

Platinum needle and loop. For most purposes finer wire is used.

tip of a sterile platinum needle (Fig. 54), and thus transfer them to a cover-glass for microscopic examination, or to new media where they may develop in pure cultures and show their growth characteristics.

In using nutrient gelatin one must always remember not to allow it to stay where the temperature is over 22° C., for if that happens the media, as a rule, will melt; nor must the liquefying colonies be allowed to grow for too long a time, or the entire media will become fluid.

Pure Cultures.—If bacteria from a colony formed from a single organism are transferred without contamination to new media, and these grow, we have what is known as a pure culture of that variety. When these are transferred to the solid media we call the growth which takes place from smearing the bacteria over the surface a surface or smear

culture, and that formed in the depth of the media by plunging the needle carrying the bacteria into it a stab culture (Figs. 55 and 56).

While transferring bacteria from one tube to another we slant the tubes so that no dust may fall within and contaminate with other bacteria the special variety we wish to transplant. The greatest care must be taken that the sterilized platinum needle used to transfer the bacteria is not infected by touching any non-sterile matter. The upper rim of culture tubes should be passed through the flame so as to destroy any bacteria resting there. Even with our utmost care bacteria will from time to time pass from the air or edges of our tubes into the culture media, and thus the possibility of contamination must always be kept in mind. When this occurs upon solid media we, as a rule, easily detect it, for we notice at some point the growth of bacteria of different colony characteristics; but in fluid media, on account of the complete mingling of the bacteria, we are not so apt to notice the additional growth.

FIG. 55

Stab cultures of three cholera spirilla in gelatin, showing in upper portion of growth considerable liquefaction of nutrient gelatin.

THE STUDY OF PURE CULTURES IN TUBED MEDIA.—A few points of the many which should be observed are the following:

Gelatin stab cultures.

A. Non-liquefying.

Line of puncture.

Filiform, uniform growth, without special characters.
Beaded, consisting of loosely placed, disjointed colonies.
Arborescent, branched, or tree-like.

Some of these points are illustrated in Fig. 56, sketched by Chester.

B. Liquefying.

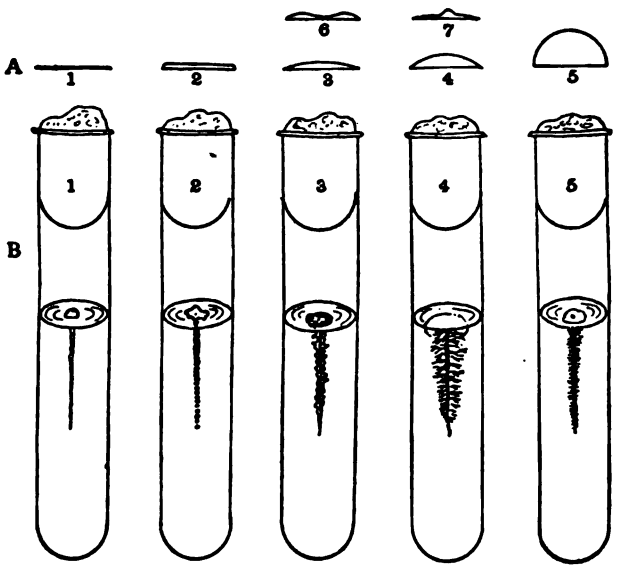
Crateriform, a saucer-shaped liquefaction of the gelatin.
Saccate, shape of an elongated sac, tubular (Fig. 55).
Statiform, liquefaction extending to the walls of the tube.

Nutrient agar tube cultures give fewer points for observation, but should be studied in the same way. The agar in the tubes is usually slanted and the culture growth is not only in the stab, but along the streaked surface. The characteristics of each should be noticed.

Opposite page 94 is appended the chart devised by a committee of the Society of American Bacteriologists comprising a set of rules and of descriptive terms to be used in giving a complete description of a bacterium. The chief advantage of using such a chart in whole or in part is that the observations of different workers may be clearly compared.

Apparatus for Obtaining a Suitable Temperature for the Growth of Bacteria.—Incubators.—In order to have a constant and proper temperature for the growth of bacteria, forms of apparatus called incubators have been devised. These consist, in their simplest form, of an inner air chamber surrounded by a double copper wall containing water (Fig. 57). The apparatus externally is lined with asbestos, to prevent radiation. It is supplied with doors and with openings for thermometers and a thermoregulator. The thermoregulators are of

FIG. 56



Showing characters of gelatin stab cultures: *A*. Characters of surface elevation: 1, flat; 2, raised; 3, convex; 4, pulvinate; 5, capitate; 6, umbilicate; 7, umbonate. *B*. Characters of growth in depth: 1, filiform; 2, beaded; 3, tuberculate-ecinulate; 4, arborescent; 5, villous. (From Chester.)

various kinds; those in most use depend upon the expansion or contraction of the fluid in the bulb *A* (Fig. 58), which rests within the water-jacket, to lessen or increase the space between the surface of the mercury *B* and the inner tube *D*, thus allowing of the passage of a greater or less quantity of gas to the burner through the tube *D*. Other forms are used in very large incubators or in incubator rooms. These usually depend upon the contraction or expansion of metal, or the use of the electric current to control the flow of the gas.

The temperature in the air chamber is kept above that of the surrounding air by means of a gas flame regulated as above described, or, when that cannot be obtained, a lamp.

When temperatures lower than that of the surrounding air are wanted, heat is reduced by passing a stream of cool water through the water chamber, which is itself regulated. When very accurate investigations are to be made a gas-pressure regulator is added to the

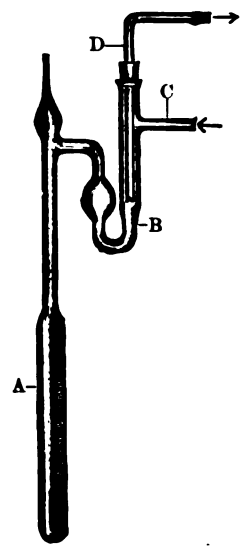
thermoregulator. Incubators may be also both warmed and regulated by electricity.

In emergencies, a culture may be developed at the blood temperature by placing it in a closed jar or bottle which is placed in a larger vessel filled with water heated at 38° C. By adding a little hot water from time to time to the outer vessel the temperature can readily be kept between 34° and 38° C., which is sufficiently uniform for bacteria such as the diphtheria bacilli to grow. A Thermos bottle will answer the purpose. As a temporary expedient during the night, when haste is necessary, it is possible, when the culture medium is solid and within a

FIG. 57

Small incubator.

FIG. 58



Thermoregulator.

strong glass tube or metal case, to make use of the body heat by putting it under the clothing next to the body or sleeping upon it. Naturally, this should only be done when other means fail.

Methods for Obtaining Anaërobic Conditions for Bacteria.— Pasteur excluded the oxygen by pouring a layer of oil on the culture fluid. A simple device is that of Koch, who placed a thin strip of sterile mica upon the agar or gelatin while still fluid in the Petri dish, after inoculation. After the solidification of the media the portion under the mica is excluded from the air and anaërobic growth can develop.

A second simple method (Liborius) is to fill the tubes with media fuller than usual and to inoculate the bacteria deep down to near the bottom of the tubes while the media are still semisolid. An anaërobic growth will take place in the lower part of the tube. In a similar way the closed arm of the fermentation tube will suffice for anaërobic growth, if the opening connecting it with the open bulb is

quite small and the medium has been freshly heated to expel any dissolved oxygen. Wright devised the following procedure: A short glass tube with constricted ends is used. Each end has a piece of rubber tubing attached. One of these is connected with a glass tube, which projects through the cotton plug of the test-tube. The test-tube contains bouillon. The whole is sterilized and then the test-tube inoculated. The bouillon is then drawn up into the constricted tube, which is sealed by simply pushing down the tube so that both rubber ends are sealed by being bent on themselves. When spores are present, a simple method suggested, I believe, by McFarland, can be successfully employed. Vessels plugged with stoppers perforated by glass tubes drawn to a point are filled to such a height that when the fluid is heated to 80° C. it will just fill them. They are inoculated when the bouillon is at about 60° C., heated to 80° C., and then sealed by closing the tube's point by means of a flame. After inoculating and heating, instead of sealing the glass tube a sterile rubber cork can be inserted.

Fig. 59

Novy jar for anaërobic cultures.

If much fermentation is expected, the cork should be clamped or tied to the bottle, so that it will not blow out. One advantage of this method is that any contaminating organisms which have no spores will be killed.

When sealed the bottles should be cooled and then placed in the incubator.

A very convenient modification of Pasteur's method for the growth of bacteria in fluid media is to cover the fluid with albolene or paraffin. In boiling, all the oxygen is driven out. We prepare all our tetanus toxins in this way: Litre flasks are filled to near the neck with bouillon. This is covered with a one-half inch layer of albolene mixed with sufficient paraffin to yield a nearly solid substance at 37° C. The bouillon after boiling is quickly cooled by setting the

Fig. 60

Buchner's anaërobic tube. The fluid consists of pyrogallic acid dissolved in 10 per cent. soda solution. By Wilson's method the tubes are charged with pieces of caustic potash covered with pyrogallic acid.

flask containing it in a shallow layer of cool water, so as to lower the temperature of the lower portion of the bouillon to 40° C. or under, while leaving the paraffin on the surface still fluid. While in this condition it is inoculated with a spore-bearing tetanus culture. Bits of tissue suspected to contain tetanus bacilli may be dropped into smaller flasks filled and prepared in the same way.

Displacement of Air.—In the more complicated methods the plates or tubes are placed in jars of a type devised by Novy (Fig. 59), in which the oxgyen is displaced by a stream of hydrogen developed by the Kipp apparatus, through the action of pure granulated zinc and a 25 per cent. solution of pure sulphuric acid. When all the oxygen has been displaced the jars are sealed by rotating the stopper.

Absorption of Oxygen.—In another method the oxygen is extracted by a mixture of pyrogallic acid and caustic potash. To each 100 c.c. of air space in the jar 1 gram of pyrogallic acid and 10 c.c. of 6 per cent. solution of potassium hydroxide are added and the jars immediately sealed. A very simple modification has been described by Wilson. In a large test-tube a small piece of solid caustic potash is placed and over this powdered pyrogallic acid is poured. This is stored until wanted. A smaller culture tube with the desired medium is inoculated. Water is now added to the large test-tube, which works its way slowly through the pyrogallic acid. The small tube is quickly inserted and the whole sealed by water or a rubber cork (Fig. 60). Solid culture media in test-tubes can be inverted over the acid soda mixture, which is then covered by a layer of albolene to prevent the absorption of oxygen from the air. The displacement method is often used along with that of absorption.

Associated with Aerobic Bacteria.—Anaërobic bacteria mixed with aërobic bacteria will frequently grow in the apparent presence of oxygen, the aërobic bacteria robbing the media of it. Thus, tetanus and diptheria grow together in an open flask of bouillon.

Method for Adapting Bacteria to Animal Fluids.—The placing of cultures in collodion sacs in the abdomens of animals has been used extensively by the Pasteur school for exalting the virulence of bacteria or trying to adapt them to species of animals differing from the one from which they were isolated.

The underlying idea is to grow the organisms in the peritoneal cavity of an animal under such conditions that the waste products of the germs will be removed, an abundant supply of nutrient material furnished, and the germs themselves protected from the action of the phagocytes. The hermetically sealed collodion sacs answer this purpose. The collodion used is the U. S. Pharmacopœia solution, which by exposure to the air has been concentrated one-third.

The sealed inoculated sacs are to be inserted into the peritoneal cavity with every possible precaution for asepsis. The sacs are left in place for days or months, as the experiment requires.

CHAPTER VI.

PRODUCTS OF BACTERIAL GROWTH.

LIGHT, HEAT, CHEMICAL COMPOUNDS, ETC.

Bacteria not only are acted upon by their surroundings, as has already been shown, but they themselves act, often markedly, upon these surroundings. We have spoken, under the effect of food upon bacteria (p. 48) of the great changes which may be produced in bacterial growths by slight changes in the food medium. So, many of the products, as noted below, are influenced to a greater or a less extent by environment.

Production of Light.—Bacteria which have the property of emitting light are quite widely distributed in nature, particularly in media rich in salt, as in sea-water, salt fish, etc. Many of these, chiefly bacilli and spirilla, have been accurately studied. The emission of light is a property of the living protoplasm of the bacteria, and is not usually due to the oxidation of any photogenic substance given off by them; at least only in two instances has such substance been claimed to have been isolated. Every agent which is injurious to the existence of the bacteria affects this property. Living bacteria are always found in phosphorescent cultures; a filtered culture free from germs is invariably non-phosphorescent; but while these organisms cannot emit light except during life, they can live without emitting light. They are best grown under free access of oxygen in a culture medium prepared by boiling fish in sea-water (or water containing 3 per cent. sea-salt), to which 1 per cent. peptone, 1 per cent. glycerin, and 0.5 per cent. asparagin are added. Even in this medium the power of emitting light is soon lost unless the organism is constantly transplanted to fresh media.

Thermic Effects.—The production of heat by bacteria does not attract attention in our usual cultures because of its slight amount, and even fermenting culture liquids with abundance of bacteria cause no sensation of warmth when touched by the hand. Careful tests, however, show that heat is produced. The increase of temperature in organic substances when stored in a moist condition, as tobacco, hay, manure, etc., is due, partly at least, to the action of bacteria.

Chemical Effects.—The changes which substances undergo as they are split up by microörganisms depend, first, on the chemical nature of the bodies involved and the conditions under which they exist, and, secondly, on the varieties of bacteria present. A complete description of these chemical changes is at present impossible. Chemists can as yet only enumerate some of the final substances evolved,

and describe, in a few cases, the manner in which they were produced. Bacteria are able to construct their body substance out of various kinds of nutrient materials, as well as to produce fermentation products or poisons; they are able to do these things either analytically or synthetically with almost equal ease. Anabolic and katabolic power exists, according to Hueppe, among bacteria to an extent known as yet among no other living things.

In the chemical building up of their body substance we can distinguish, as Hueppe concisely puts it, several groups of phenomena: Polymerization, a sort of doubling up of a simple compound; synthesis, a union of different kinds of simple compounds into one or more complex substances; formation of anhydride, by which new substances arise from a compound through the loss of water; and reduction or loss of oxygen, which is brought about especially by the entrance of hydrogen into the molecule. The breaking down of organic bodies of complicated molecular structure into simpler combinations takes place, on the other hand, through the loosening of the bonds of polymerization, through hydration or entrance of water into the molecule, and through oxidation.

The chemical effects which take place from the action of bacteria are greatly influenced by the presence or absence of free oxygen. The access of pure atmospheric oxygen makes the life processes of most bacteria more easy, but is not indispensable when available substances are present which can be broken up with sufficient ease. The standard of availability is very different for different bacteria.

In the presence of oxygen some of the decomposition products that are formed by the attack of the anaërobic bacteria are further decomposed and oxidized by the aërobes; they are thereby rendered, as a rule, inert and consequently harmless as well as odorless in most cases. Some bacteria have adapted themselves to the exclusive use of combined oxygen, using those compounds from which oxygen can be obtained, and others—the obligatory aërobes—are able to live only in the presence of free oxygen. The facts of anaërobiosis are of great importance to technical biology and to pathology. Many parasitic bacteria are found to produce far more poison in the absence of air than in its presence. The following four types of chemical activity can be separated: 1. Production of substances which help in some way the life of the cell. These substances may be secreted and retained within the cell, or liberated from it; *e.g.*, ferments or enzymes; true toxins (?). 2. Production of substances liberated by the bacteria as waste products. 3. Production of substances by the breaking down of the food media; *e.g.*, putrefactive products, due largely to enzyme action. 4. The production of substances which help form the protoplasm of the bacterial cell itself.

Fermentation.—The term fermentation is differently used by different authors. Some call every kind of decomposition due to microorganisms or their products a fermentation, speaking thus of the putrefactive fermentation of albuminous substances; others limit the term

to the process when accompanied by the visible production of gas; others, again, take fermentation to mean only the decomposition of carbohydrates, with or without gas-production.

Fermentation may be defined as a chemical decomposition of an organic compound, induced by the life processes of living organisms (organized ferments), or by chemical substances thrown off from the organisms (unorganized or chemical ferments or enzymes). In the first case the action is due to the life processes necessary for the growth of the organisms producing the ferment, as in the formation of acetic acid from alcohol by the action of the vinegar plant; in the second case the enzyme, either within or outside of the organism and having no direct connection with the growth of the organism, causes a structural change without losing its identity, as in digestion. E. Buchner (*Berichte d. Deutsch. chem. Gesellsch.*, xxx., 117–124 and 1110–1113) has shown that, even in those cases of fermentation in which formerly it was believed the organized cell itself was necessarily concerned, the cell protoplasm squeezed from crushed cells and separated by filtration is able to cause the same changes as the organized cells. This brings fermentation by unorganized and organized ferments very closely together, the one being a substance thrown off from the cell, the other a substance ordinarily retained within the cell. The elaboration of both ceases with the death of the bacteria producing them. Fermentation, therefore, requires the living agent or its enzyme. It furthermore demands the proper nutriment, temperature, and moisture and the absence of deleterious substances.

Fermentation yields products that are poisonous to the ferment; hence fermentation ceases when the nutriment is exhausted or the fermentation is in excess. Often, however, the process will begin again after diluting the fermented medium, showing that the *concentration* of the harmful products plays an important part in the inhibitory action.

Specific names are applied to various well-known fermentations according to the product—*e. g.*, *acetic*, yielding acetic acid; *alcoholic* or *vinous*, yielding alcohol; *ammoniacal*, yielding ammonia; *amylic*, yielding amylic alcohol; *benzoic*, yielding benzoic acid; *butyric*, yielding butyric acid; *lactic*, yielding lactic acid; and *viscous*, yielding a gummy mass.

Characteristics of Ferments or Enzymes.—Ferments are non-dialyzable. They withstand moderate dry heat, but are usually destroyed in watery solutions on exposure of 10 to 30 minutes to a temperature of 60 to 70° C. They are injured by acids, especially the *inorganic* ones, but are resistant to all alkalies. They, even when present in the most minute quantities, have the power of splitting up or decomposing complex organic compounds into simpler, more easily soluble and diffusible molecules. The changes thus made may greatly aid in rendering the food stuff suitable for bacterial growth. A simple example of bacterial fermentation of carbohydrates produced by an enzyme is that of grape-sugar:

$$\underset{\text{Grape-sugar}}{C_6H_{12}O_6} = \underset{\text{2 Alcohol.}}{2C_2H_6O} + \underset{\text{2 Carbon dioxide.}}{2CO_2}$$

Or,

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{2 Lactic acid.}}{2C_3H_6O_3}$$

Or,

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{3 Acetic acid.}}{3C_2H_4O_2}$$

Far less common is oxidizing fermentation, such as occurs, for example, in the production of acetic acid from alcohol. Here the energy is acquired not from the decomposition, but by the oxidation of the alcohol.

The Proteolytic Ferments.—The proteolytic ferments which are somewhat analogous to trypsin—being capable of changing albuminous bodies into soluble and diffusible substances—are very widely distributed. The liquefaction of gelatin, which is chemically allied to albumin, is due to the presence of a proteolytic ferment or trypsin. The production of proteolytic ferments by different cultures of the same variety of bacteria varies considerably—far more than is generally supposed. Even among the freely liquefying bacteria, such as the cholera spirillum and the staphylococcus, poorly liquefying strains have been repeatedly found. These observations have taught us that gelatin cultures must be observed for at least one month before deciding that no liquefaction will occur. Most conditions which are unfavorable to the growth of bacteria seem to interfere also with their liquefying power.

Bitter-tasting products of decomposition may be formed by certain liquefying bacteria in media containing proteid, as, for example, in milk.

Diastatic Ferments.—Diastatic ferments convert starch into sugar. This action is demonstrated by mixing starch paste with suitable cultures to the resulting mixture of which thymol has been added, and keeping the digestion for six to eight hours in the incubating oven; then, on the addition of Fehling's solution and heating, the reaction for sugar appears—the reddish-yellow precipitate due to the reduction of the copper.

Inverting Ferments.—Inverting ferments (that is, those which convert polysaccharides into monosaccharides) are of very frequent occurrence. Bacterial invertin withstands a temperature of 100° C. for more than an hour, and is produced in culture media free from proteid. The presence or absence of such a ferment is often an important means of differentiating between closely related varieties of organisms. For more details as to the action of ferments on sugars see chapter on the colon-typhoid groups.

Rennin-like Ferments.—Rennin-like ferments (substances having the power of coagulating milk with neutral reaction, independent of acids) are found not infrequently among bacteria. The *B. prodigiosus*, for instance, in from one to two days coagulates to a solid mass milk which has been sterilized at 55° to 60° C.

Alkaline Products and the Fermentation of Urea.—Aërobic bacteria always produce alkaline products from albuminous substances. Many species also produce acids from sugars, which explains the fact that neutral or slightly alkaline broth often becomes acid at first from the fermentation of the sugar contained in the meat used for making the media. When the sugar is used up the reaction often becomes alkaline, as the production of alkalies continues. The substances producing the alkalinity in cultures are chiefly ammonia, amine, and the ammonium bases.

The conversion of urea into carbonate of ammonia affords an example of the production of alkaline substances by bacteria:

$$\underset{\text{Urea.}}{CO(NH_2)_2} + \underset{\text{2 Water.}}{2H_2O} = \underset{\text{Ammonium carbonate.}}{CO_3(NH_4)_2}$$

The power of decomposing urea is not widespread among bacteria.

Pigment Production.—Pigments have no known importance in connection with disease, but are of interest and have value in identifying bacteria. Their chemical composition is not generally known.

Red and Yellow Pigments.—Of the twenty-seven red and yellow chromogenic bacteria studied by Schneider, almost all produce pigments soluble in alcohol and insoluble in water. The large majority of these pigments possess in common the property of being colored blue-green by sulphuric acid and red or orange by a solution of potash. Though varying considerably in their chemical composition and in their spectra, they may be classified, for the most part, among that large group of pigments common to both the animal and vegetable kingdoms known as *lipochromes*, and to which belong the pigments of fat, yolk of eggs, the carotin of carrots, turnips, etc.

Violet Pigments.—Certain bacteria produce violet pigments, also insoluble in water and soluble in alcohol, but insoluble in ether, benzol, and chloroform. These are colored yellow when treated in a dry state with sulphuric acid, and emerald-green with potash solution.

Blue Pigments.—Blue pigments, such as the blue pyocyanin produced by *B. pyocyaneus;* the fluorescent pigment common to many so-called fluorescent bacteria is different (bacteriofluorescence). In cultures the pigment is at first blue; later, as the cultures become alkaline, it is green.

Numerous investigations have been made to determine the cause of the variation in the chromogenic function of bacteria. All conditions which are unfavorable to the growth of the bacteria decrease the production of pigment, as cultivation in unsuitable media or at too low or too high a temperature, etc. The *B. prodigiosus* seldom makes pigment at 37° C., and when transplanted at this temperature, even into favorable media, the power of pigment production is gradually lost. *B. pyocyaneus* does not produce pigment under anaërobic conditions.

Ordinarily colorless species of bacteria sometimes produce pigments. Occasionally colored and uncolored colonies of the same species of bac-

teria may be seen to occur side by side in one plate culture, as, for example, in the case of staphylococcus pyogenes.

Ptomains.—Nencki, and later Brieger, Vaughan, and others, succeeded in isolating organic bases of a definite chemical composition out of putrefying fluids—meat, fish, old cheese, and milk—as well as from pure bacterial cultures. Some of these were found to exert a poisonous effect, while others were harmless. The poisons may be present in the decomposing cadaver (hence the name ptomain, from πτῶμα, putrefaction), and, in consequence, have to be taken into consideration in questions of legal medicine. They may be formed also in the living human body, and, if not made harmless by oxidation, may come to act therein as self-poisons or leucomains. They possess the characteristics of alkaloid bodies and are different from the specific poisonous toxins.

Many ptomains are known already and among them are some whose exact chemical constitution is established. Especially interesting is the substance cadaverin, which was separated by Brieger from portions of decomposing dead bodies and from cholera cultures, by reason of the fact that Ladenburg prepared it synthetically and showed it to be pentamethylenediamin $[(NH_2)_2(CH_2)_5]$. The cholin group is particularly interesting. Cholin itself ($C_5H_{15}NO_2$) arises from the hydrolytic breaking-up of lecithin, the fat-like substance found in considerable amounts in the brain and other nervous tissue. By the oxidation of cholin there can be produced the highly toxic muscarin, found by Schmiedeberg in a poisonous toadstool and isolated by Brieger in certain decomposing substances:

$$\underset{\text{Cholin}}{C_5H_{15}NO_2} + O = \underset{\text{Muscarin}}{C_5H_{15}NO_3}$$

The ptomain tyrotoxicon was obtained from cheese, milk, and ice-cream by Vaughan.

Pyocyanin ($C_{14}H_{14}N_2O$), which produces the color of blue or blue-green pus, is a ptomainic pigment. Similar bodies of a basic nature may be found in the intestinal contents as the products of bacterial decomposition. Some of these are poisons and can be absorbed into the body. Since the name ptomain was given to the poisonous products of bacterial growth before these products were chemically understood it is by many wrongly applied to all poisons found in food. Such poisoning may be due to true toxins or even living bacteria.

The isolation of these substances can here be only briefly referred to. According to Brieger's method, which is the one now generally employed, the cultures having a slight acid reaction (HCl) are boiled down, filtered, and the filtrate concentrated to a syrupy consistency, dissolved in 96 per cent. alcohol, purified and precipitated by means of an alcoholic solution of bichloride of mercury.

The Bacterial Toxins.—Any poisonous substance formed in the growth of bacteria or other microörganism may be called a toxin.

The different bacterial toxins vary greatly in their characteristics. As little is known concerning their chemical nature, we are not able to classify them. There are certain known differences among them which are important and which may be made use of for purposes of study to divide the bacteria into two groups:

1. Those varieties of bacteria that excrete in ordinary culture media water-soluble very specific toxic products, *extracellular toxins*. Type: diphtheria, tetanus.

2. Those varieties which possess apparently only *endotoxins*, that is, true toxins which are more or less closely bound to the living cell, and which are only in a small degree separable in unchanged condition outside of the body. On death of the cell they partly become free, partly remain united, or become secondary poisonous modifications, no longer of the nature of toxins. Type: cholera, typhoid, pneumococcus.

Among the intracellular poisons some are heat resistant. To these the name proteins is frequently given.

Extracellular Toxins.—Among the properties of the extracellular toxins are the following: They are, so far as known, uncrystallizable, and thus differ from ptomains; they are soluble in water and they are slowly dialyzable, through thin membranes, but not through thick membranes such as are used in refining antitoxic globulins; they are precipitated along with proteids by concentrated alcohol, 65 per cent. or over, and also by ammonia sulphate; if they are proteids they are either albumoses or allied to the albumoses; they are relatively unstable, having their toxicity diminished or destroyed by heat as well as by chemical manipulation (the degree of heat, etc., which is destructive varies much in different cases). Their potency is often altered in the precipitations practised to obtain them in a pure or concentrated condition, but among the precipitants ammonium sulphate has but moderate harmful effect. A remarkable characteristic of the group is that they are highly specific in their properties and have the power in the infected body to excite the production of antitoxins. The diphtheria and tetanus bacilli are the best known extracellular toxin producers.

Precipitation of Extracellular Toxins.—Ammonium sulphate crystals are added to the fluid containing the toxin until it is saturated. A large excess of ammonium sulphate crystals is then added and the whole kept at about 37° C. for twelve to eighteen hours. The toxin is precipitated and rises to the surface. This is skimmed off and dried in a vacuum or in an exsiccator containing strong sulphuric acid. The dried powder is placed in vacuum tubes and stored in the dark. Under these conditions the toxins deteriorate very slowly. During the process there may be a considerable loss of toxin, even when every care is taken. Tetanus toxin is especially liable to deterioration. With the toxin other substances are precipitated. The diphtheria toxin is best precipitated from the bouillon by adding alcohol sufficient to produce a 65 per cent. solution.

The precipitate is removed from the alcohol by filtration with the least possible delay.

Intracellular Toxins.—Regarding the intracellular toxins which are more intimately associated with the bacterial cell and are produced by all bacteria we know much less, but it is probable that their chemical nature is somewhat similar, though they differ in their resistance to heat,—*e. g.*, some of the toxins elaborated by tubercle bacilli withstand boiling, while others do not. In the case of all toxins the fatal dose for an animal varies with the body weight, age, and general conditions.

Ferment Characteristics of Toxins.—The comparison of the action of bacteria in the tissues in the production of these toxins to what takes place in the gastric digestion has raised the question of the possibility of the elaboration by these bacteria of ferments, by which the process may be started. It would not be prudent to dogmatize as to whether the toxins do or do not belong to such an ill-defined group of substances as the ferments. It may be pointed out, however, that the essential concept of ferments is that of a body which can originate change without itself being appreciably changed, and no evidence has been adduced that toxins fulfil this condition. Another property of ferments is that, so long as the products of fermentation are removed, the action of a given amount of ferment is indefinite. In the case of toxins no evidence of such an occurrence has been found. A certain amount of a toxin is always associated with a given amount of disease effect.

Similar Vegetable and Animal Poisons.—Substances similar to the bacterial endotoxin ferments and soluble toxins are formed by many varieties of cells other than bacteria. The ricin and abrin poisons obtained from the seeds of the *Ricinus communis* and the *Abrus precatorius* have a number of properties similar to those of the diphtheria and tetanus poisons. The active poisons contained in ricin and abrin have not yet been isolated, but the impure substances are extremely poisonous. When injected into suitable animals anti-poisons are produced and accumulate in the serum. These neutralize the poisons wherever they come in contact with them.

They resemble the toxins in a general way in the manner in which they react to heat and chemicals. They are precipitated by alcohol. Through animal membranes they are less dialyzable than albumoses. Substances having these characteristics are called toxalbumins.

Poisonous snakes secrete poisons which have many of the characteristics of the bacterial albumoses. The venom contains some substances similar to peptone and others similar to globulin. The former cause general nervous symptoms and paralysis of the respiratory center, while the latter cause intense local reaction with hemorrhages around the point of injection. The injection of venins into animals is followed by the production of antivenins which neutralize the venins. When the serum containing abundant suitable antevenins is injected into an infected person it has considerable therapeutic value.

Ehrlich's Theories as to the Nature of Extracellular Toxins.—From a large number of most carefully conducted experiments with the toxin and antitoxin of diphtheria, Ehrlich has formulated a theory concerning the former. This theory has undergone several modifications since it was first proposed, and it is difficult to give an exact statement of its present status. Generally speaking, however, in condensed form its essential points are as follows:

Toxins and antitoxins neutralize one another after the manner of chemical reagents. The chief reasons for this belief lie in the observed facts: (*a*) that neutralization takes place more rapidly in concentrated than in dilute solutions, and (*b*) that warmth hastens and cold retards neutralization. From these observations Ehrlich concludes that toxins and antitoxins act as chemical reagents do in the formation of double salts. A molecule of the poison requires an exact and constant quantity of the antitoxin in order to produce a neutral or harmless substance. This implies that a specific atomic group in the toxin molecule combines with a certain atomic group in the antitoxin molecule.

The toxins, however, are not simple bodies, but easily split into other substances which differ from one another in the avidity with which they combine with antitoxin.

These derivatives Ehrlich calls prototoxins, deuterotoxins, and tritotoxins.

All forms of toxins are supposed to consist of two modifications, which combine in an equally energetic manner with antitoxin or with suitable substance in the cells, but differ in their resistance to heat and other destructive agents.

The less resistant form passes readily into a substance called toxoid which has the same affinity for the antitoxin as the original toxin, but is not poisonous. The facts observed, Ehrlich thinks, are best explained on the supposition that the toxic molecule contains two independent groups of atoms, one of which may be designated as the haptophorous and the other as the toxophorous group. It is by the action of the former that toxin unites with antitoxin or cell molecule and allows the latter to exert its poisonous effect.

The toxophorous group is unstable, but after its destruction the molecule still unites with the antitoxin or the sensitive molecule through its retained haptophorous group.

Bordet has shown that toxin unites in different multiples with antitoxin, so that the toxin molecule may have its affinity slightly, partly, or wholly satisfied by antitoxin. Slightly satisfied, it is still feebly toxic; combined with a larger amount of antitoxin, it is not toxic; but still may, when absorbed into the system, lead to the production of antitoxin. Fully saturated, it has no poisonous properties and no ability to stimulate the production of antitoxin.

The most important of the extracellular toxins are those produced by the diphtheria and tetanus bacilli. These are very powerful; 0.0000001 gram of the dried filtrate of a tetanus culture will frequently

kill a white mouse, while 100 times of that amount of dried diphtheria filtrate has killed a guinea-pig.

The same bacterium may produce several entirely distinct toxins, thus, according to Madsen and Ehrlich, the specific tetanus poison consists of two toxins, tetanospasmin and tetanolysin. To the first of these the tetanic convulsions are due, while the second has a hæmolytic action.

Altogether different from the poison effects are the immunization processes produced by the cell substances of bacteria, whether they be obtained from bacterial bodies or from chemical preparations. These processes have little or nothing to do with the toxic action of the cell proteids, but rather depend upon the introduction of suitable receptors, that is, substance capable of union with the molecules of the cells which give rise to the antibodies.

The pyogenic action of their proteids is common to all bacteria, this depending principally upon their being extraneous albuminous substances. Pyogenic effects may be produced in like manner by extraneous albumins of non-bacterial origin. That every extraneous albuminous substance is harmful to the organism which seeks to resist its action is shown by those specific precipitating ferments, the precipitins, which are produced in the organisms after the introduction of every extraneous albumin.

Reduction Processes.—The following processes depend wholly or in part upon the reducing action of nascent hydrogen.

1. Sulphuretted Hydrogen (H_2S). All bacteria, according to Petri and Maassen, possess the power of forming sulphuretted hydrogen, particularly in liquid culture media containing much peptone (5 to 10 per cent.); only a few bacteria form H_2S in bouillon in the absence of peptone, while about 50 per cent. in media containing 1 per cent. peptone possess the property of converting sulphur into sulphuretted hydrogen, for which purpose is required the presence of nascent hydrogen. The presence of H_2S is determined by placing a piece of paper moistened with lead acetate inside the neck of the flask containing the culture, closing the mouth with a cotton-wool stopper, and over this again an india-rubber cap (black rubber free from sulphur). The paper is colored at first brownish and later black; repeated observation is necessary, as the color sometimes disappears toward the end of the reaction. Apparently negative results should not be rashly accepted as conclusive.

2. The reduction of blue litmus pigments, methylene blue, and indigo to colorless substances. The superficial layer of cultures in contact with the air shows often no reduction, only the deeper layers being affected. By agitation with access of air the colors may be again restored, but, at the same time, if acid has been formed, the litmus pigment is turned red.

3. The reduction of nitrates to nitrites, ammonia, and free nitrogen. The first of these properties seems to pertain to a great many bacteria.

The test for nitrites is made as follows: Two bouillon tubes containing nitrates are inoculated, and, along with two uninoculated tubes, are allowed to remain in the incubator for several days; then to the cultures and control test is added a small quantity of colorless iodide of starch solution (thin starch paste containing 0.5 per cent. potassium iodide) and a few drops of pure sulphuric acid. The control tubes remain colorless or become gradually slightly blue, while if nitrites are present a dark blue or brown-red coloration is produced. A test may be made also by sulfonilic acid and α naphthylamin hydrochloride, which give a brown-red coloration proportional to the amount of nitrite present.

The demonstration of ammonia is made by the addition of Nessler's reagent to culture media free from sugar. In bouillon, if ammonia be present, Nessler's reagent is almost immediately reduced to black mercurous oxide. A strip of paper saturated with the reagent can also be suspended over the bouillon tube, or this can be distilled at a low temperature with the addition of magnesium oxide and the distillate treated with Nessler's reagent. A yellow to red coloration indicates the presence of ammonia. Controls are necessary. Place 1 c.c. of bouillon and 49 c.c. NH_3 free H_2O in Nessler jar with controls. Add reagent to each, allow to stand fifteen minutes and read color which is compared with standards.

Aromatic Products of Decomposition.—Many bacteria produce aromatic substances as the result of their growth. The best known of these are indol, skatol, phenol, and tyrosin. Systematic investigations have only been made with regard to the occurrence of indol and phenol.

Test for Indol.—To a bouillon culture, which should, if possible, be not under eight days old and free from sugar, is added half its volume of 10 per cent. sulphuric acid. If in heating to about 80° C. a pink or bluish-pink coloration is immediately produced it indicates the presence of both indol and nitrites, the above-described nitroso-indol reaction requiring the presence of both of these substances for its successful operation. This is the so-called "cholera-red reaction," but it may be applied to many other spirilla besides cholera and to certain bacilli also. As a rule, however, the addition of sulphuric acid alone is not sufficient, and a little nitrite must be added; this may be done later, the culture being first warmed without nitrite, when, if there is no reaction or a doubtful one, 1 to 2 c.c. of 0.005 per cent. solution of sodium nitrite is added until the maximum reaction is obtained. The addition of strong solutions of nitrite colors the acid liquid brownish-yellow and ruins the test. Out of sixty species examined by Lehmann, twenty-three gave the indol reaction.

Decomposition of Fats.—Pure melted butter is not a suitable culture medium for bacteria. The rancidity of butter is brought about (1) as the result of a purely chemical decomposition of the butter by the oxygen of the air under the influence of sunlight, and (2) through the formation of lactic acid from the milk-sugar left in the butter. Fats are, however, attacked by bacteria when mixed with gelatin and used as culture media, with the consequent production of acid.

Putrefaction.—By putrefaction is understood in common parlance every kind of decomposition due to bacteria which results in the production of malodorous substances. Scientifically considered, putrefaction depends upon the decomposition of albuminous substances,

which are frequently first peptonized and then further decomposed. Typical putrefaction occurs only when oxygen is absent or scanty; the free passage of air through a culture of putrefactive bacteria—an event which does not take place in natural putrefaction—very much modifies the process: first, biologically, as the anaërobic bacteria are inhibited, and then by the action of the oxygen on the products or by-products of the aërobic and facultative anaërobic bacteria.

As putrefactive products we have peptone, ammonia, and amines, leucin, tyrosin, and other amido substances; oxyfatty acids, indol, skatol, phenol, ptomains, toxins, and, finally, sulphuretted hydrogen, mercaptans, carbonic acid, hydrogen, and, possibly, marsh-gas.

Nitrifying Bacteria.—According to recent observations, nitrification is produced by a special group of bacteria, cultivated in the laboratory with difficulty, which do not grow on our usual culture media. From the investigations of Winogradsky it would appear that there are two common microörganisms present in the soil, one of which converts ammonia into nitrites and the other converts nitrites into nitrates.

Conversion of Nitrous and Nitric Acids into Free Nitrogen.—This process is performed by a number of bacteria.

The practical importance of these organisms is that by their action large quantities of nitrates in the soil, and especially in manure, may become lost as plant food by being converted into nitrogen.

By the aid of certain root bacteria, which gain entrance to the roots of legumes and there produce nodular formations, the leguminous plants are enabled to assimilate nitrogen from the atmosphere. It is not known exactly how this assimilation of nitrogen occurs, but it is assumed that the zoöglœa-like bacteria, called *bacteroids*, constantly observed in the nodules, either alone or in a special degree, possess the property of assimilating and combining nitrogen. It seems, moreover, to have been recently established that, independently of the assistance of the legumes, certain nodule bacteria exist free in the soil, which accumulate nitrogen by absorbing it from the air. These various nitrifying, denitrifying, and nitrogen-fixing bacteria are described in detail in the special chapter upon bacteria in nature.

Formation of Acids from Carbohydrates.—Free acids are formed by many bacteria in culture media containing some form of sugar or other fermentable carbohydrates, such as the alcohol mannite; the production of acid in ordinary bouillon takes place on account of the presence of meat-sugar, which is usually derived in small quantities from the meat.[1] According to Theobald Smith, all anaërobic or facultative anaërobic bacteria form acids from sugar; the strict aërobic species do not, or do so very slowly that the acid is concealed by the almost simultaneous production of alkali. The formation of acid occurs sometimes with and sometimes without the production of gas. Excessive acid production may cause the death of the bacteria from the increase in acidity of the culture media.

[1] According to Theobald Smith, 75 per cent. of the beef ordinarily bought in the markets contains appreciable quantities of sugar (up to 0.3 per cent.).

If after the sugar is consumed, not enough acid has been formed to kill the bacteria, the acid is neutralized gradually and in the end the reaction becomes less acid or even alkaline.

Among the acids produced the most important is lactic acid; also traces of formic acid, acetic acid, propionic acid, and butyric acid, and not infrequently some ethyl-alcohol and aldehyde or acetone are formed. Occasionally no lactic acid is present, and only the other acids are formed.

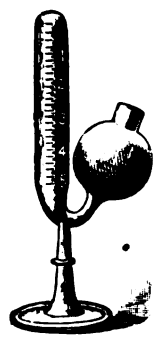
Various bacteria, as yet incompletely studied, possess the property of producing butyric acid and butyl-alcohol from carbohydrates.

Some bacteria also seem to have the power of decomposing cellulose.

Formation of Gas from Carbohydrates and Other Fermentable Substances of the Fatty Series.—The only gas produced in *visible* quantity in sugar-free culture media is nitrogen. If sugar is vigorously decomposed by bacteria, as long as pure lactic acid or acetic acid is produced there may be no development of gas, as, for instance, with the *B. typhosus* on grape-sugar; but frequently there is much gas developed, especially in the absence of air. About one-third of the acid-producing species also develop gas abundantly, this consisting chiefly of CO_2, which is always mixed with H. Marsh-gas is seldom formed by bacteria, with the exception of those decomposing cellulose.

In order to test the production of gas, a culture medium composed of solid or semi-solid nutrient agar, containing about 1 per cent. glucose, lactose, or other carbohydrate, may be used. At the end of eight to twelve hours in the incubator (or twenty-four hours' room temperature) the agar will be seen to be full of gas-bubbles or broken up into holes and fissures.

FIG. 61



Fermentation tube.

For the determination of the quantity and kind of gas produced by a given microörganism the fermentation tube recommended by Theobald Smith is the best. This is a bent tube, constricted greatly at its lowest portion, supported upon a glass base, as shown in Fig. 61. Fermentation tubes should have the following essential points: The neck should be narrow, to prevent as far as possible the diffusion of gas; this is particularly necessary to prevent the entrance of oxygen which would of course destroy the anaërobiosis. The bulb should be large enough to hold *all* the fluid in the vertical arm together with the amount normally in the bulb itself. The tube is filled with a culture medium consisting of peptone bouillon (without air bubbles) to which 1 per cent. of glucose, lactose, or other sugar has been added, and sterilized in the steam sterilizer. It is then inoculated with a loopful of a culture of the organism in question, and observations taken:

1. If there is a turbidity produced in the open bulb it indicates the presence of an aërobic species; if this clouding occurs only in the closed arm, while the open bulb remains clear, it is an anaërobic species.

2. The quantity of gas produced daily should be marked on the

upright arm; if the tube is graduated a note of it is taken and the percentage calculated on the fourth to the sixth day after gas production has ceased.

3. A rough analysis of the gas produced may be made as follows: Having signified by a mark on the tube the quantity of gas produced, the open bulb is completely filled with a 10 per cent. solution of soda, the mouth tightly closed with the thumb, and the mixture throughly shaken. After a minute or two all the gas is allowed to rise to the top of the closed arm by inclining and turning the tube, and then, removing the thumb, the volume of gas left after the union of the NaOH with the CO_2 is noted. The remainder is nitrogen, hydrogen, and marsh-gas. If it is desired to test for the presence of hydrogen, the thumb is again placed over the open end and the gas collected under it. As the thumb is moved a lighted match is brought in contact with the gas. If hydrogen is present a slight explosion occurs.

Formation of Acids from Alcohol and Other Organic Acids.—It has long been known that the *Bacterium aceti* and allied bacteria convert dilute solutions of ethyl-alcohol into acetic acid by oxidization:

$$\begin{array}{l} CH_3 \\ | \\ CH_2OH \end{array} + O_2 = \begin{array}{l} CH_3 \\ | \\ COOH. \end{array} + H_2O.$$

The higher alcohols—glycerin, dulcit, mannite, etc.—are also converted into acids.

Finally, numerous results have been obtained from the conversion of the fatty acids and their salts into other fatty acids by bacteria. As a rule, the lime-salts of lactic, malic, tartaric, and citric acids have been employed, these being converted into various acids by the action of bacteria, as, for example, butyric, propionic, valerianic, and acetic acids; also succinic acid, ethyl-alcohol, and, more rarely, formic acid have been produced. The gases formed were chiefly CO_2 and H.

Thus Pasteur found that anaërobic bacteria convert lactate of lime into butyric acid.

Important Characteristics which should be Noted in the Complete Study of a Bacterium.—The accompanying descriptive chart which gives the points decided upon by the Society of American Bacteriologists (1907) as necessary for the complete identification of an organism is inserted in order to insure unity of methods and thus make comparative studies easier. Some pathogenic bacteria require special media for their growth; moreover, they do not need testing with all of the tests mentioned in this chart in order to identify them. With some varieties the cultural characteristics are of the greatest importance, while with others pathogenic or toxic effects occupy the chief place.

Source..... Date of Isolation..... Name

DETAILED FEATURES.

NOTE—Underscore required terms. Observe notes and glossary of terms on opposite side of card.

I. MORPHOLOGY (1)

1. **Vegetative Cells**, Medium used.....
temp.....age.....days
Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
Limits of Size.....
Size of Majority.....
Ends, *rounded, truncate, concave.*

Agar	Orientation (grouping).....	
	Chains (No. of elements).....	
Hanging-block	*Short chains, long chains*	
	Orientation of chains,	*parallel, irregular.*

2. **Sporangia**, medium used.....temp.....
age.....days
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of Size.....Size of Majority.....

Agar	Orientation (grouping).....	
	Chains (No. of elements).....	
Hanging-block	Orientation of chains,	*parallel, irregular.*

Location of Endospores, *central, polar.*
3. **Endospores.**
Form, *round, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching.*
4. **Flagella**, No.....Attachment *polar, bipolar, peritrichiate.* How Stained.....
5. **Capsules**, present on.....
6. **Zoogloeæ**, Pseudozoogloeæ.
7. **Involution Forms**, on.....in....days at....° C.
8. **Staining Reactions.**
1 : 10 watery fuchsin, gentian-violet, carbol-fuchsin, Lœffler's alkaline methylene-blue.
Special Stains
Gram.....Glycogen.....
Fat.....Acid-fast.....
Neisser.....

II. CULTURAL FEATURES (2)

1. **Agar Stroke.**
Growth, *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Optical characters, *opaque, translucent, opalescent, iridescent,*
Chromogenesis (3).....
Odor, *absent, decided, resembling*.....
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*
2. **Potato.**
Growth *scanty, moderate, abundant, transient, persistent.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (3).....Pigment in water *insoluble, soluble;* other solvents.....
Odor, *absent, decided, resembling*.....
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium, *grayed, browned, reddened, blued, greened.*
3. **Loeffler's Blood-serum.**
Stroke *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (3).....
Medium *grayed, browned, reddened, blued, greened.*
Liquefaction begins in.....d. complete in.....d,
4. **Agar Stab.**
Growth *uniform, best at top, best at bottom;* surface growth *scanty, abundant; restricted, wide-spread.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent; liquefaction.*
5. **Gelatin Stab.**
Growth *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate plumose, arborescent.*
Liquefaction *crateriform, napiform, infu form, saccate, stratiform;* begins in..... complete in.....d.
Medium *fluorescent, browned*.....
6. **Nutrient Broth.**
Surface growth, *ring, pellicle, flocculent, mem none.*
Clouding *slight, moderate, strong; transie sistent; none; fluid turbid.*
Odor, *absent, decided, resembling*.....
Sediment, *compact, flocculent, granular, flak on agitation, abundant, scant.*
7. **Milk.**
Clearing without coagulation.
Coagulation *prompt, delayed, absent.*
Extrusion of whey begins in .....day
Coagulum *slowly peptonized, rapidly peptonize*
Peptonization begins on....d. complete on
Reaction, 1d...., 2d...., 4d...., 10d....,
Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*
8. **Litmus Milk.**
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow r
9. **Gelatin Colonies.**
Growth *slow, rapid.*
Form, *punctiform, round, irregular, ameboi lioid, filamentous, rhizoid.*
Elevation, *flat, effuse, raised, convex, p crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*
10. **Agar Colonies.**
Growth *slow, rapid,* (temperature.....
Form, *punctiform, round, irregular, ameboi lioid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, striate.*
Elevation, *flat, effuse, raised, convex, p umbonate.*
Edge, *entire, undulate, lobate, erose, lacer briate, floccose, curled.*
Internal structure, *amorphous, finely-, granular, grumose, filamentous, floccose,*
11. **Starch Jelly.**
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained.....
12. **Silicate Jelly (Fermi's Solution).**
Growth *copious, scanty, absent.*
Medium stained.....
13. **Cohn's Solution.**
Growth *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*
14. **Uschinsky's Solution.**
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*
15. **Sodium Chloride in Bouillon.**
Per cent. inhibiting growth.....
16. **Growth in Bouillon over Chloroform,** *unr feeble, absent.*
17. **Nitrogen.** Obtained from *peptone, asparagi coll, urea, ammonia salts, nitrogen.*
18. **Best media for long-continued growth**.....
.....
19. **Quick tests for differential purposes**.....
.....
.....

III. PHYSICAL AND BIOCHEMICAL FEAT

1. Fermentation-tubes containing peptone-water or sugar-free bouillon and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit
Gas production, in per cent. $\left(\frac{H}{CO_2}\right)$						
Growth in closed arm						
Amount of acid produced 1d.						
Amount of acid produced 2d.						
Amount of acid produced 3d.						

Group No. (1)

2. **Ammonia production**, *feeble, moderate, strong, absent, masked by acids.*
3. **Nitrates in nitrate broth.**
   Reduced, not reduced.
   Presence of nitrites.....ammonia.....
   Presence of nitrates.....free nitrogen.....
4. **Indol production**, *feeble, moderate, strong.*
5. **Toleration of Acids**, *great, medium, slight.*
   Acids tested.....
6. **Toleration of NaOH**, *great, medium, slight.*
7. **Optimum reaction for growth** in bouillon, stated in terms of Fuller's scale .....
8. **Vitality on culture media**, *brief, moderate, long.*
9. **Temperature relations.**
   Thermal; death-point (10 minutes' exposure in nutrient broth when this is adapted to growth of organism).....C.
   Optimum temperature for growth.....C.; or best growth at 15° C., 20° C., 25° C., 30° C., 37° C., 40° C., 50° C., 60° C.
   Maximum temperature for growth.....C.
   Minimum temperature for growth .....C.
10. **Killed readily by drying**: resistant to drying.
11. **Per cent. killed by freezing** (salt and crushed ice or liquid air).....
12. **Sunlight**: Exposure on ice in thinly sown agar plates; one-half plate covered (time 15 minutes), *sensitive, not sensitive.*
    Per cent. killed.....
13. **Acids produced**.....
14. **Alkalies produced**.....
15. **Alcohols**.....—....
16. **Ferments**, *pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab, etc.*.....
    .....
17. **Crystals formed**:.....
18. **Effect of germicides:**

Substance	Method used	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. Pathogenic to Animals.
   Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea-pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.....
2. Pathogenic to Plants:
   .....
   .....
   .....
3. **Toxins**, *soluble, endotoxins.*
4. **Non-toxin forming.**
5. **Immunity bactericidal.**
6. **Immunity non-bactericidal.**
7. **Loss of virulence on culture-media**: *prompt, gradual, not observed in*.....months.

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

MORPHOLOGY (2)

- Diameter over 1μ
- Chains, filaments
- Endospores
- Capsules
- Zoogloea. Pseudozoogloea
- Motile
- Involution forms
- Gram's Stain

CULTURAL FEATURES (3).

Broth
- Cloudy, turbid
- Ring
- Pellicle
- Sediment

Agar
- Shining
- Dull
- Wrinkled
- Chromogenic

Gel. Plate
- Round
- Proteus-like
- Rhizoid
- Filamentous
- Curled

Gel. Stab
- Surface-growth
- Needle-growth

Potato
- Moderate, absent
- Abundant
- Discolored
- Starch destroyed

- Grows at 37° C.
- Grows in Cohn's Sol.
- Grows in Uschinsky's Sol.

BIOCHEMICAL FEATURES

Liquefaction
- Gelatin (4)
- Blood-serum
- Casein

Milk
- Acid curd
- Rennet curd
- Casein peptonized

- Indol (5)
- Hydrogen sulfid
- Ammonia (6)
- Nitrates reduced (5)
- Fluorescent
- Luminous

DISTRIBUTION

- Animal pathogen, epizoon
- Plant pathogen, epiphyte
- Soil
- Milk
- Fresh water
- Salt water
- Sewage
- Iron bacterium
- Sulfur bacterium

CHAPTER VII.

THE SOIL BACTERIA AND THEIR FUNCTIONS—AIR BACTERIA —BACTERIA IN INDUSTRIES.

The bacteria[1] in the soil belong to many varieties. Some varieties are only accidentally present, being due to the contamination of the earth with the bacteria contained in animal fæces and other waste products. The majority, however, pass their life and reproduce themselves chiefly or wholly in the soil. Many of these varieties have most important functions to perform in continuing the earth's food supply. Without them plant food; and, therefore, animal food, would cease to exist. Some make available for plants, the carbon, nitrogen, hydrogen, and other compounds locked up in the dead bodies of animals and plants. Others construct food for plants from the gases of the air and the inorganic elements of the earth which in their simpler forms were not available.

The bacteria together with the other somewhat less important microscopic plants and animals, thus form a vital link in the earth's life cycle, plants and animals. The bacteria in the soil require for their activities food, moisture, and a proper temperature. They may be present to the extent of many millions in a single gram of rich loam, while in an equal quantity of sand they may be almost absent.

The various species associated together in the soil flora influence each other. Thus anaërobic bacteria are enabled to grow because of associated aërobes using up the free oxygen, while other species make assimilable substances not usable by others.

The Splitting up of Carbon Compounds.—The plants form starch, and from it cellulose, wood, fats, and sugar. These substances once formed cannot be utilized by other generations of plants. Some of these are transformed in the bodies of animals, but the largest percentage await the activities of the microörganisms. The sugars and starches usually undergo an alcoholic fermentation, excited by the yeasts and moulds with the production of alcohol and carbon-dioxide, or an acid fermentation excited by bacteria with the production of acids and frequently of carbon-dioxide.

Cellulose which is so resistant to decay is attacked by certain varieties of bacteria which are abundant in the soil. They act both in the presence and absence of free oxygen. Moulds also act on cellulose. Carbon-dioxide, marsh gas, and other products are produced. Wood is apparently first attacked by the fungi and only later by the micro-organisms. These bacteria are carried into the intestines and act upon cellulose and other substances.

[1] L. H. Bailey. "Bacteria in Relation to Country Life."

The Decomposition of Nitrogenous Compounds.—Plants obtain their nitrogen chiefly in the form of nitrates. The small amount of usable nitrogen in the soil must be constantly replenished. This must either come from the nitrogen forming a part of proteid materials or from the free nitrogen in the air.

The animals utilize the plant proteids and reduce them to much simpler compounds, such as urea, but even these are not suitable for plant use. We now know that microörganisms are employed to break compounds into simpler compounds and also to utilize the nitrogen of the air.

Decomposition.—This process is to some extent carried out through the agency of yeasts, moulds, and fungi, but it is chiefly due to the activities of bacteria. When this process is carried on in the absence of oxygen it is incomplete giving rise to substances with unpleasant odors, such as H_2S, NH_3 and CH_4. This is called putrefaction. When oxygen is freely accessible more complete decomposition occurs with such end products as CO_2,N and H_2O. These two processes, putrefaction and complete decay, cannot be sharply separated as the second usually follows the first. The varieties of organisms causing these changes are many. Some groups will be found chiefly in decaying vegetable substances, others in animal tissues. They include all morphologic forms of bacteria as well as yeasts and higher fungi. These forms exist everywhere in nature, although in various degrees, so that every bit of dead organic matter is sure to be decomposed if only moisture and warmth are present. *B. subtilis* and *B. proteus vulgarius* are well known laboratory bacteria that are commonly found among decomposing materials. *B. proteus* is described under pathogenic bacteria. *B. subtilis* (hay bacillus) has the following characteristics (Fig. 62).

Source and Habitat.—Hay, straw, soil, dust, milk, etc.

Morphology.—Short, thick rods with round ends, sometimes form threads. Sometimes also chains of long rods, short rods, and coccus forms. 0.8 to 1.2μ broad, 1.3 to 3μ long. Often united in strings and threads.

Staining Reaction.—Stains by Gram's method.

Capsule, Flagella, Motility.—Bacillus posseses a thin capsule and many flagella which are long and numerous; short forms actively motile; threads immotile.

Spore Formation.—Oval spores formed in presence of air germinating at right angles to long diameter. Spores are set free in about 24 hours, size 1.2 by 0.6μ; widely distributed in nature, dust, air, excreta, etc., (see Fig. 62).

Biology: Cultural Characters (Including Biochemical Features).—Bouillon.—Uniformly cloudy growth with marked pellicle, wrinkled and thick; copious spore formation.

Gelatine Plates and Tubes.—Saucer-like depressions; colonies have granular centres and folded margins. Surface growth in stab cultures is whitish-gray; colonies sink on liquefaction of medium; liquefaction progresses in a cylindrical form, and a thick white scum is formed.

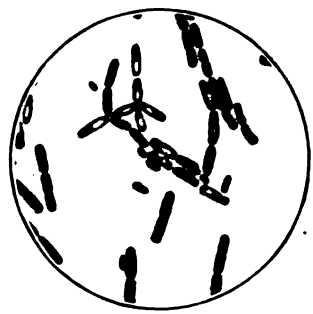
Agar Plates and Tubes.—Small, irregular, grayish-white colonies; moist glistening growth along needle track in stab cultures.

The bacteria in taking certain atoms from the molecules utilized in their growth leave the other atoms to enter into new relations and

form new compounds. The actual products will depend on the decaying substance, the variety of bacteria and the conditions present.

Nitrification.—This is a process of oxidation by which through bacterial activities ammonia compounds are changed to nitrates and thus rendered utilizable by plants. This change is accomplished in two stages; first, the ammonia is oxidized to nitrite and second to nitrate. The nitrates are taken up by the plant roots from the soil. The bacterial nature of these changes were discovered in 1877 by two French investigators, Schlosing and Muntz. They noted that fermenting sewage after a time lost its ammonia and gained in nitrates, but that if the sewage was treated with antiseptics, so that fermentation ceased, no such change occurred. Warrington first and Winogradsky later more thoroughly investigated the bacterial cause of these changes. The latter by means of silica jelly, which contained no organic matter, was able to isolate two varieties of cocci, one in Europe and the other in America, which were able to change ammonia to nitrites. He called the one nitrosomonas and the other nitrosococcus. They are capable of acting on almost any ammonia salt. One variety of organisms capable of changing nitrites to nitrates was isolated, and this bacillus he called nitrobacter. These are small slightly elongated bacilli. These bacteria are remarkable in that in pure cultures very small amounts of organic matter in the media act as antiseptics. They appear to be able to depend on mineral substances for their food. These bacteria are extremely important, for the plants take up most of their nitrogen in the form of nitrates. These changes are mostly produced in the surface soil. If the reaction of the soil becomes acid growth ceases. Soil bacteriologists are studying the nitrifying power of different types of soil under identical conditions. The process being one of oxidation, the access of air is necessary.

FIG. 62



Bacillus subtilis with spores. Agar culture. Stained with gentian violet. × 1000 diameters. (Fraenke.)

Denitrification.—This is a reducing process. The nitrate is made to yield up a part or all of its oxygen and thus becomes changed to nitrites and to ammonia and even to free nitrogen. The partial change does not rob the soil of its available nitrogen as does the total change, for the nitrites and ammonia may be changed by the nitrifying bacteria to nitrates. These bacteria exist normally in most soils and are especially abundant in manure. There are three different types of nitrogen reduction: 1. The reduction of nitrates to nitrites and ammonia. 2. The reduction of nitrates and nitrites to gaseous oxides of nitrogen. 3. The reduction of nitrites with the development of free nitrogen gas.

Nitrogen Fixing Bacteria.—Helbrigel in 1886 demonstrated that certain plants were able to use the nitrogen of the air and this ap-

parently through the aid of bacteria growing in their roots. These root bacteria are named *B. radicicola.* They produce enlargements (tubercles) on the roots.

According to Ball,[1] there is no reasonable doubt but that *B. radicicola* can and usually does remain active for very long periods in soil devoid of leguminous vegetation. Furthermore, the bacterium diffuses at a very considerable rate through soils that are in proper condition; therefore, if a soil should be found lacking the organism, it is illogical to attempt to introduce it artificially without having first made the soil fit for the development of the bacteria.

It has not been shown by anyone that increased powers of resistance to unfavorable conditions of certain varieties are at all correlated with their enhanced "greed for nitrogen." Moreover, it is far from being proven that any one race or "physiologic species" is really more virile than another. Greig-Smith[2] has shown that as many as three races are sometimes present in one and the same tubercle. Possibly, therefore, fixation of nitrogen may occur most rapidly only when two or more of these races are growing together.

Buchanan[3] has recently made a minute morphologic study of *B. radicicola.* Some of his conclusions are as follows:

1. Considerable variation in the morphology of *B. radicicola* may be induced in artificial media by the use of appropriate nutrients. Of the salts of the organic acids, sodium succinate brings about the most luxuriant development and the production of the greatest variety of bacteroids.

2. *B. radicicola* in the roots of the legumes shows the same type of bacteroids as may be found in suitable culture media. On the other hand, there is little or no correspondence between the type of bacteroid produced in culture media by a certain organism and that produced in the nodule by the same form.

3. It is probable that the term *B. radicicola* includes an entire group of closely related varieties or species which differ from each other to some degree in morphological characters.

4. The nodule organism resembles morphologically both the yeasts and the bacteria. The difference between this form and those ordinarily included under the terms Bacillus and Pseudomonas justify the use of a separate generic name, *Rhizobium.*

In 1893 Winogradsky furnished proof that there are in the soil bacteria which are outside of the plant roots performing the same function as those within the roots. These bacilli he called *Clostridium pasteurianum.* They are anaërobic and produce spores. Their power to fix nitrogen is increased in presence of sugar and lessened in presence of nitrogenous substances.

Beyerinck in 1901 described two aërobic species of nitrogen fixing

[1] Ball, O. M. A contribution to the Life History of B. radicicola Beij. Centralbl. f. Bakt., etc., 1909, II. Abt., xxiii, 47.

[2] Greig-Smith. Journ. Soc. Chem. Indust., 1907, No. 7.

[3] Buchanan, R. E. The Bacteroids of Bacillus radicicola. Centralbl. f. Bakt., etc., 1909, II. Abt., xxiii, 59.

bacteria. Later Bailey described three additional species. These were called *Azotobacter*. These studies have already led to the inoculation of soils and to the investigation of the kind of soils and crops best fitted for the growth of these bacteria. Many impoverished soils have already been greatly improved. There are probably many other varieties of bacteria capable of fixing nitrogen, because one can hardly examine the roots of any leguminous plants, without finding tubercles different. The use of seed inoculated with the special variety of bacteria suitable for the plant and the soil is already largely practised.

Bacteria and Soil Minerals.—Some of the bacterial products act upon the inorganic constituents of the soil. The carbonic dioxide and the organic acids act upon compounds of lime and magnesia, practically insoluble in water, to form more soluble substances. The same is true of the rock phosphates, the silicate of potassium, sulphates, etc.

Scientific farming is beginning to make use of the knowledge already acquired, and there is reason to hope that great practical advantages will flow from the investigation of the relation of bacteria to soil exhaustion and replenishment.

The effect of excessive bacterial development appears at times to be harmful to the soil. Each crop seems to favor the growth of certain varieties, and the exhaustion of the soil which follows the constant raising of the same crop is now suspected to be due in part at least to the continuance of a few restricted species of bacteria in the soil, which failing to produce all the necessary substances for the nutrition of the special crop, vegetation suffers, or again the bacteria finally entirely dissipate substances already in the soil necessary to growth.

The application of manure not only adds food for plant life, but also countless numbers of bacteria which make the food more available. The greatest number of bacteria are contained a little below the surface of the soil, where they are protected from drying and sunlight and are in contact with oxygen and with the roots and other food of the superficial soil.

Bacteria in Sewage.—The materials which flow from our sewers are a menace to public health, mainly because they so frequently contain pathogenic bacteria. The other products of men and animals are offensive but rarely concentrated enough in drinking water to be appreciably deleterious. Sewage can be made harmless by being sterilized, but can be freed from offense only by the destruction of organic matter. This, except when chemical precipitants are used, is almost wholly obtained through bacterial processes. The purifying value of soil has long been recognized. This is largely due to the action of the soil bacteria.

In 1895, the Englishman, Cameron, introduced the "septic tank" which was a covered cemented pit. The sewage admitted at the bottom flowed out at the top, after about twenty-four hours' subjection to anaërobic conditions. The anaërobic bacteria during this time ferment the organic matter energetically, liquefy it, and develop abundant gas.

The knowledge that soil and sand filters act not only mechanically, but also and perhaps chiefly bacteriologically, having been acquired, intermittent soil filtration was established as one of the best means of bacteriologically purifying sewage. The sewage is conducted to the beds, allowed to pass through, and then after a few hours again poured on. This purification is based chiefly on the action of the aërobic bacteria in the upper layers of the soil or sand. The best practical results are obtained by combining the two processes, first the anaërobic treatment is used to break down the solid materials, and then the intermittent sand filtration, to oxidize the compounds and render these products harmless. With low temperatures the chemical changes are very much lessened and the filter beds act more as pure mechanical filters. The anaërobic bacteria change the proteid substances into simple chemical compounds, among which is ammonia. The carbohydrates are changed into gaseous compounds, acids, etc. The gases are mainly nitrogen, carbon-dioxide and marsh-gas. The bacterial changes produced in sewage poured on contact beds made of coarse coke, clinkers, or other material act much as in the sand filters after the filtration.

Varieties of Bacteria in Filter Beds and Septic Tanks.—The septic tanks all contain spore-bearing bacilli, which destroy cellulose, others that attack nitrogenous compounds. The cocci are in a minority. The filter beds have a number of small non-spore-bearing bacilli, some of these change ammonia into nitrites and nitrates. There are also denitrifying bacteria. As before mentioned, the bacterial efficiency of the bed is increased with suitable temperature and much lessened with low temperature.

Sewage Farming.—The action of bacteria is availed of in disposing of sewage over fields. The amount of sewage which can be poured on a certain area is limited. One acre of land can usually take care of the sewage from one hundred persons. If too much is poured on, it runs off impurified or clogs the soils and prevents the access of oxygen to aërobic bacteria. In warm weather evaporation and bacterial activities are much greater than in cold weather. So far as experience shows, those who eat vegetables from these small farms contract no disease from them.

Bacteria in Atmosphere.—The air is kept constantly in motion by winds so that fine particles are constantly being carried into it from the ground, especially in an inhabited area with its dusty streets. The rays of sunlight visibly reveal these particles to us. The bacteria in the dust of the fields and streets are carried along with these dust particles. They are usually the harmless soil bacteria or the almost equally harmless intestinal bacteria of animals. Pathogenic human bacteria happen rarely to be carried in harmful numbers except under exceptional circumstances and usually as spores, such as those of anthrax bacilli from the dust from the wool and hides of infected animals or of tetanus bacilli from the infected manure. After a storm few bacteria are in the air, while on a dry windy day many thousands

exist in a cubic meter. In warm weather rain carries down the bacteria of the air. The bacteria in the air of the country are much less than in the city air. Forests decrease the number of bacteria.

On high mountains and on the sea far from land bacteria are very scarce. The bacteria that multiply in the soil of street and country are almost entirely saprophytic types. Sunlight and drying rapidly destroy bacteria. In dwellings the bacterial content depends on many factors, of which the chief are the opening of windows to the outside dust-laden air, the cleanliness of the dwelling, and the amount of stirring up of the dust by sweeping. It is almost impossible to separate the effect of the bacteria which we inhale from that of the dust particles which they accompany. Both probably act as slight irritants and so predispose to definite infections.

Bacteria in Industries.—The curing of tobacco is apparently due partly to bacterial processes and partly to the action of leaf enzymes.

The preservation of foods against decomposition by bacteria, yeasts, moulds, and higher fungi is obtained by using processes which will prevent the growth of microörganisms. Drying, exposure to wood smoke with consequent absorption of creosote, the addition of salt and sugar, of acids such as vinegar, spices, germicides such as boracic acid, formaldehyde, all are familiar methods of making foods unsuitable for bacterial growth. Instead of using food preserved by drying or chemicals, products may be kept at temperatures too low for bacterial growth. Cold storage of meats, eggs, vegetables, etc., is now common.

The sterilization of food substances by heat with protection from infection afterward is made use of extensively in the canning of fruits and vegetables. Care must be taken that absolutely all bacteria are killed, for otherwise decomposition will finally occur.

Vinegar Making.—Vinegar is made from some weak alcoholic solution by the union of alcohol with oxygen. This oxidation can be brought about by a purely chemical process. When vinegar is formed in the usual way bacteria are essential. The scum on the surface of the fermenting alcohol is a mass of microörganisms. The mother of vinegar was named mycoderma by Pearson. Kutzing showed that this was composed of living cells. Hansen proved these to be bacteria. We now know there are many varieties of bacilli capable of producing this fermentation. Each variety has its own optimum temperature and differs in the amount of acid it produces. Most of these have the peculiarity of growing at high temperatures into long threads without any traces of division. At low temperatures they produce long threads with swollen centres. The usual vinegar is made by using the variety of bacilli prevalent in the surroundings, but the custom is growing of adding to the pasteurized alcoholic solution the special variety desired in pure culture.

Sauerkraut.—This is cabbage leaves shredded, slightly fermented, and prevented from decay by the lactic acid bacteria. At first both yeasts and bacteria increase together, but with the increase in acidity

all growth ceases. Putrefaction is prevented by the same cause. The lactic acid bacteria are the same as those found in sour milk.

Ensilage.—The fermentation here is believed to be due partly to enzymes in the corn tissues and partly to bacterial action. The first changes are due chiefly to the enzymes.

The Bacterial Disease of Plants.—These are probably as serious and varied for plants as for animals. The pear blight, the wilt disease of melons, the brown rot of tomatoes, the black rot of cabbages are examples. These plant diseases can be communicated by means of the pathogenic pure cultures of bacteria experimentally just as readily as animal diseases by their specific bacteria.

Bacterial Fermentation in Relation to Miscellaneous Products.—Pasteur in 1857 explained the process of fermentation as due to the action of microörganisms. He demonstrated that the change of sugar into lactic acid only occurred when living bacilli were present. If the fluid was sterilized the fermentation ceased. He stated that "organic liquids do not alter until a living germ is introduced into them." When the action is direct we speak of an organized ferment; when it is indirect, that is, due to the cell product, we call it an unorganized soluble ferment or enzyme. Similar enzymes are produced by the cells of the animal tissues, such as ptyalin, pepsin, and trypsine. Pasteur's work led to the conclusion that the different fermentations were due to different varieties of organisms. The major part of fermentation is due to yeast.[1] Some important fermentations are due to bacteria and a few to the moulds.

Wines and Beers.—Alcoholic Fermentation.—If there is a development of the yeast cells in a solution of grape-sugar we have a fermentation of the sugar with a final development of alcohol and carbon-dioxide. It is thus that beers and wines are developed. When the carbohydrate is in the form of starch this is first converted into sugar and then later into the final products. If the sugar is in the form of saccharose, it is first changed by the yeast ferments to glucose. In all these three forms of fermentation the sugar is changed into alcohol and carbonic acid. When the alcohol reaches about 13 per cent. it stops further fermentation. These yeasts called saccharomyces comprise a number of distinct varieties, some of which are cultivated while others, called "wild yeasts," propagate themselves. The distillery, brewery, and wine industries each make use of special yeasts and special conditions. The rising of bread is one of the most common uses of fermentation by yeast. The yeast acts upon the sugar made by the diastase from the starch. The resulting CO_2 and alcohol creates myriads of little bubbles in the dough.

Diseases in Beer and Wines.—Hansen, Pasteur, and others demonstrated that the spoiling of beers and wines was due to the development of varieties of bacteria and yeasts which produce different kinds of fermentation from that desired. These produce alterations in flavor, bitterness, acidity.

[1] For further study of yeasts see Sec. II.

CHAPTER VIII.

THE DESTRUCTION OF BACTERIA BY CHEMICALS—PRACTICAL USE OF DISINFECTANTS.

MANY substances, when brought in contact with bacteria, combine with their cell substance and destroy the life of the bacteria. While in the vegetative stage bacteria are much more easily killed than when in the spore form, and their life processes are inhibited by substances less deleterious than those required to destroy them.

Bacteria, both in the vegetative and in the spore form, differ among themselves considerably in their resistance to the poisonous effects of chemicals. The reason for this is not wholly clear, but it is connected with the structure and chemical nature of their cell substance.

Chemicals in sufficient amount to destroy life are more poisonous at temperatures suitable for the best growth of bacteria than at lower temperatures, and act more quickly upon bacteria when they are suspended in fluids singly than when in clumps, and in pure water rather than in solutions containing organic matter. The increased energy of disinfectants at higher temperatures indicates in itself that a true chemical reaction takes place. In estimating the extent of the destructive or inhibitive action of chemicals the following degrees are usually distinguished:

1. The growth is not permanently interfered with, but the pathogenic and zymogenic functions of the organism are diminished—*attenuation.* This loss of function is usually quickly recovered.

2. The organisms are not able to multiply, but they are not destroyed—antiseptic action. When transferred to a suitable culture fluid free of the disinfectant these bacteria are capable of reproduction.

3. The vegetative development of the organisms is destroyed, but not the spores—incomplete or complete sterilization or disinfection, according as to whether spores are present in the organisms exposed and as to whether these spores are capable of causing infection.

4. Vegetative and spore formation are destroyed. This is complete *sterilization* or *disinfection.*[1]

The methods employed for the determination of the germicidal action of chemical agents on bacteria are, briefly, as follows:

If it is desired to determine the minimum concentration of the chemi-

[1] Disinfection strictly defined is the destruction of all organisms and their products which are capable of producing disease. Sterilization is the destruction of all saprophytic as well as parasitic bacteria. It is not necessary in most cases to require disinfectants to be capable of sterilizing infected materials containing spores, for there are but few varieties of pathogenic bacteria which produce spores.

cal substance required to produce complete inhibition of growth we proceed thus: A 10 per cent. solution of the disinfectant is prepared and 1 c.c., 0.5 c.c., 0.3 c.c., 0.1 c.c., etc., of this is added to 10 c.c. of liquefied gelatin, agar, or bouillon, or, more accurately 10 c.c. minus the amount of solution added, in so many tubes. The tubes then contain 1 per cent., 0.5 per cent., 0.3 per cent., and 0.1 per cent. of the disinfectant. The fluid media in the tubes are then inoculated with a platinum loopful of the test bacteria. The melted agar and gelatin may be simply shaken and allowed to remain in the tubes, and watched as to whether any growth takes place, or the contents of the tubes may be poured into Petri dishes, where the development or lack of development of colonies and the number can be observed. If no growth occurs in any of the dilutions, higher dilutions are tested. Bacteria that have been previously injured in any way will be inhibited by much weaker solutions of chemicals than will vigorous cells. The same test can be made with material containing only spores.

If it is desired to determine the degree of concentration required for the destruction of vegetative development, the organism to be used is cultivated in bouillon, and into each of a series of tubes is placed a definite amount of diluted culture from which all clumps of bacteria have been filtered; to these a definite amount of watery solution of different percentages of the disinfectant is added. At intervals of one, five, ten, fifteen, and thirty minutes, one hour, and so on a small platinum loopful of the mixture is taken from each tube and inoculated into 10 c.c. of fluid agar or gelatin, from which plate cultures are made. Whenever it is possible that the antiseptic power of the bacteria approaches somewhat the germicidal, it is necessary to inoculate a second series of tubes from the first so as to decrease still further the amount of antiseptic carried over. The results obtained are signified as follows: x per cent. of the disinfectant in watery solution and at x temperature kills the organism in twenty minutes, y per cent. kills in one minute, and so on. If there be any doubt whether the trace of the disinfectant carried over with the platinum loops may have rendered the gelatin unsuitable for growth, thus falsifying results, control cultures should be made by adding bacteria which have been somewhat enfeebled by slight contact with the disinfectant to fluid to which a similar trace of the disinfectant has been added. If the strength of the disinfectant is to be tested for different substances it must be tested in these substances or their equivalent, and not in water.

The disinfectant to be examined should always be dissolved in an inert fluid, such as water; if, on account of its being difficultly soluble in water, it is necessary to use alcohol for its solution, control experiments may be required to determine the action of the alcohol on the organism. Sometimes, as in the case of corrosive sublimate, the chemical unites with the cell substance to form an unstable compound, which inhibits the growth of the organism for a time before destroying it. If this compound is not broken up in the media, it will probably not be in the body. In some tests it is of interest to break up this union

and note then whether the organism is alive or dead. With corrosive sublimate the bacteria die in fifteen to thirty minutes after the union occurs.

In the above determinations the absolute strength of the disinfectant required is considerably less when culture media poor in albumin are employed than when the opposite is the case. Cholera spirilla grown in bouillon containing no peptone or only 0.5 per cent. of peptone are destroyed in half an hour by 0.1 per cent. of hydrochloric acid; grown in 2 per cent. peptone-bouillon, their vitality is destroyed in the same time on the addition of 0.4 per cent. HCl. In any case the organisms to be tested should all be treated in exactly the same way and the results accompanied by a statement of the conditions under which the tests were made. It is becoming the custom to state the power of a disinfectant in terms of comparison with pure carbolic acid. A substance which had the same destructive power in a 1 to 1000 solution as carbolic acid in a 1 to 100 solution would be rated as of a strength ten times that of carbolic acid.

The following table gives the results and methods used in an actual experiment to test the effect of blood serum upon the disinfecting action of bichloride of mercury and carbolic acid upon bacteria:

TEST FOR THE DIFFERENCE OF EFFECT OF BICHLORIDE OF MERCURY AND CARBOLIC ACID SOLUTIONS ON TYPHOID BACILLI IN SERUM AND IN BOUILLON.

Time	1′	3′	5′	10′	20′	30′	45′	1 hr.	1½ hrs.	2 hrs.	
A. Serum.....2.5 c.c. $HgCl_2$ sol. 1:1000 2.5 c.c. Typhoid broth culture.	+	+	+	−	−	−	−	−	−	−	Solution equals 1:2000 bichloride.
B. Bouillon....2.5 c.c. $HgCl_2$ sol. 1:1000 2.5 c.c. Typhoid broth culture.	−	−	−	−	−	−	−	−	−	−	Same.
C. Serum.....2.5 c.c. Carbolic sol. 5% 2.5 c.c. Typhoid broth culture.	+	+	−	−	−	−	−	−	−	−	Solution equals 2½% carbolic acid.
D. Bouillon....2.5 c.c. Carbolic sol. 5% 2.5 c.c. Typhoid broth culture.	+	−	−	−	−	−	−	−	−	−	Same.

− Indicates total destruction of bacteria with no growth in media.
+ Indicates lack of destruction of bacteria with growth in media.

Many substances which are strong disinfectants become altered under the conditions in which they are used, so that they lose a portion or all of their germicidal properties; thus, quicklime and milk of lime act by means of their alkali and are disinfecting agents only so long as sufficient calcium hydroxide is present. If this is changed by the carbon dioxide of the air into carbonate of lime it becomes harmless. Bichloride of mercury and many other chemicals form compounds

with many organic and inorganic substances, which, though still germicidal, are much less so than the original substances. Solutions of chlorine, peroxides, etc., when in contact with an excess of organic matter soon become inert because of the chemical compounds formed.

The Disinfecting Properties of Inorganic Compounds.—Bichloride of Mercury.—This substance, which dissolves in 16 parts of cold water, when present in 1 part in 100,000 in nutrient gelatin or bouillon, inhibits the development of most forms of bacteria. In water 1 part in 50,000 will kill many varieties in a few minutes, but in bouillon twenty-four hours may be needed. With organic substances its power is lessened, so that 1 part to 1,000 may be required. Most spores are killed in 1 500 watery solution within one hour. Corrosive sublimate is less effective as a germicide in alkaline fluids containing much albuminous substance than in watery solution. In such fluids, besides loss in other ways, precipitates of albuminate of mercury are formed which are at first insoluble, so that a part of the mercuric salt does not really exert any action. In alkaline solutions, such as blood, blood serum, pus, sputum, tissue fluids, etc., the soluble compounds of mercury are converted into oxides or hydroxides.

For ordinary use, where corrosive sublimate is employed, solutions of 1:500 and 1:2000 will suffice, when brought in contact with bacteria in that strength, to kill the vegetative forms within from one to twenty minutes, the stronger solution to be used when much organic matter is present.

Mercuric chloride volatilizes slowly and it is better to wash off walls after use of bichloride solutions. Solutions of this salt should not be kept in metal receptacles. Mercuric chloride solution has disadvantages in that it corrodes metals, irritates the skin, and forms almost inert compounds with albuminous matter. In order to avoid accidents, solutions of this odorless disinfectant should be colored by some dye.

Biniodide of Mercury.—This salt is very similar in its effect to the bichloride.

Nitrate of Silver.—Nitrate of silver in watery solution has about one-fourth the value of the bichloride of mercury as a disinfectant, but nearly the same value in inhibiting growth. In albuminous solutions it is equal to bichloride of mercury. Compounds of silver nitrate and albuminous substances have been used because of the absence of irritative properties combined with moderate antiseptic power.

Sulphate of Copper.—This salt has about 50 per cent. of the value of mercuric chloride. It has a quite remarkable affinity for many species of algæ, so that when in water 1:1,000,000 it destroys many forms; 1:400,000 destroys typhoid bacilli in twenty-four hours when the water has no excessive amount of organic material. It is not known to be poisonous in this strength, so that it can be temporarily added to water supplies.

Sulphate of Iron.—This is a much less powerful disinfectant than sulphate of copper. A 5 per cent. solution requires several days to

kill the typhoid bacilli. It can only be considered as a mild antiseptic and deoderant.

Zinc Chloride.—This is very soluble in water, but is a still weaker disinfectant than copper sulphate.

Sodium Compounds.—A 30 per cent. solution of NaOH kills anthrax spores in about ten minutes, and in 4 per cent. in about forty-five minutes. One per cent. kills vegetative forms in a few minutes. Sodium carbonate kills spores with difficulty even in concentrated solution, but at 85° C. it kills spores in from eight to ten minutes. It is used frequently to cover metallic instruments. A 5 per cent. solution kills in a short time the vegetative forms of bacteria. Even ordinary soapsuds have a slight bactericidal as well as a marked cleansing effect. The bicarbonate has almost no destructive effect on bacteria.

Calcium Compounds.—Calcium hydroxide, $Ca(OH)_2$, is a powerful disinfectant; the carbonate, on the other hand, is almost without effect. The former is prepared by adding one pint of water to two pounds of lime (quicklime, CaO). Exposed to the air the calcium hydrate slowly becomes the inert carbonate. A 1 per cent. watery solution of the hydroxide kills bacteria which are not in the spore form within a few hours. A 3 per cent. solution kills typhoid bacilli in one hour. A 20 per cent. solution added to equal parts of fæces or other filth and mixed with them will completely sterilize them within one hour.

Effect of Acids.—An amount of acid which equals 40 c.c. of normal hydrochloric acid per litre is sufficient to prevent the growth of all varieties of bacteria and to kill many. Twice this amount destroys most bacteria within a short time. The variety of acid makes little difference. Bulk for bulk, the mineral acids are more germicidal than the vegetable acids, but that is because their molecular weight is so much less. A 1:500 solution of sulphuric acid kills typhoid bacilli within one hour. Hydrochloric acid is about one-third weaker, and acetic acid somewhat weaker still. Citric, tartaric, malic, formic, and salicylic acids are similar to acetic acid. Boric acid destroys the less resistant bacteria in 2 per cent. solution and inhibits the others.

Gaseous Disinfectants.—The germicidal action of gases is much more active in the presence of moisture than in a dry condition.

Sulphur Dioxide (SO_2).—Numerous experiments have been made with this gas owing to the fact that it has been so extensively used for the disinfection of hospitals, ships, apartments, clothing, etc. This gas is a much more active germicide in a moist than in a dry condition; due, no doubt, to the formation of the more active disinfecting agent—sulphurous acid (H_2SO_3). In a pure state anhydrous sulphur dioxide does not destroy spores, and is not certain to destroy bacteria in the vegetative form. Sternberg has shown that the spores of the *Bacillus anthracis* and *Bacillus subtilis* are not killed by contact for some time with liquid SO_2 (liquefied by pressure). Koch found that various species of spore-bearing bacilli exposed for ninety-six hours in a disinfecting chamber to the action of SO_2, in the proportion of from 4 to

6 per cent. by volume, were not destroyed. In the absence of spores, however, the anthrax bacillus in a moist condition, attached to silk threads, was found by Sternberg to be destroyed in thirty minutes in an atmosphere containing 1 volume per cent.

As the result of a large number of experiments with SO_2 as a disinfectant it has been determined that an "exposure for eight hours to an atmosphere containing at least 4 volumes per cent. of this gas *in the presence of moisture*" will destroy most, if not all, of the pathogenic bacteria in the absence of spores. Four pounds of sulphur burned for each 1000 cubic feet will give an excess of gas.

Peroxide of Hydrogen (H_2O_2).—This is an energetic disinfectant, and in 2 per cent. solution (about 40 per cent. of the ordinary commercial article) will kill the spores of anthrax in from two to three hours. A 20 per cent. solution of a good commercial hydrogen peroxide solution will quickly destroy the pyogenic cocci and other spore-free bacteria. It combines with organic matter, becoming inert. It is prompt in its action and not poisonous, but apt to deteriorate if not properly kept.

Chlorine.—Chlorine is a powerful gaseous germicide, owing its activity to its affinity for hydrogen and the consequent release of nascent oxygen when it comes in contact with microörganisms in moist condition. It is, therefore, a much more active germicide in the presence of moisture than in a dry condition. Thus, Fischer and Proskauer found that dried anthrax spores exposed for an hour in an atmosphere containing 44.7 per cent. of dry chlorine were not destroyed; but if the spores were previously moistened and were exposed in a moist atmosphere for the same time, 4 per cent. was effective, and when the time was extended to three hours 1 per cent. destroyed their vitality. The anthrax bacillus, in the absence of spores, was killed by exposure in a moist atmosphere containing 1 part to 2500 for twenty-four hours.

In watery solutions 0.2 per cent. kills spores within five minutes and the vegetative forms almost immediately.

Chlorinated Lime (Called "Chloride of Lime").—Chlorinated lime is made by passing nascent chlorine gas over unslaked lime. It should not contain less than 10 per cent. of available chlorine, and can now be obtained containing 30 per cent. It should have a strong odor of chlorine. Its efficacy depends on the chlorine it contains in the form of hypochlorites. The calcium hypochlorite is readily broken up into hypochlorous acid. A solution in water of 0.5 to 1 per cent. of chlorinated lime will kill most bacteria in one to five minutes, and 1 part in 100,000 will destroy typhoid bacilli in twenty-four hours. A 5 per cent. solution usually destroys spores within one hour. Chlorinated lime not only bleaches, but destroys fabrics.

The Hypochlorites (Labarraque's Solution).—Solutions of hypochlorites are practically the same as solutions of chlorinated lime and are much more expensive.

Bromine and *iodine* are of about the same value as chlorine for gaseous disinfectants, in the moist condition; but, like chlorine, they

are not applicable for general use in house disinfection, owing to their poisonous and destructive properties; they have a use in sewers and similar places.

Trichloride of iodine in 0.5 per cent. solution destroys the vegetative forms of bacteria in five minutes.

Organic Disinfectants.—Alcohol in 10 per cent. solution inhibits the growth of bacteria; absolute alcohol kills bacteria in the vegetative form in from several to twenty-four hours. According to Epstein, 50 per cent. alcohol (in water) has more germicidal power than any other strength, the power gradually diminishing with both stronger and weaker solutions.

Formaldehyde.—Formaldehyde, or formic aldehyde, was isolated by von Hoffmann in 1867, who obtained it by passing the vapors of methyl-alcohol mixed with air over finely divided platinum heated to redness. The methyl-alcohol is oxidized and produces formaldehyde as follows:

$$CH_3OH + O = CH_2O + H_2O.$$

Formaldehyde is a gaseous compound possessed of an extremely irritating odor. At a temperature of 68° F. the gas is polymerized—that is to say, a second body is formed, composed of a union of two molecules of CH_2O. This is known as a paraformaldehyde, and is a white, soapy body, soluble in boiling water and in alcohol. Formaldehyde is sold in commerce as a clear, watery liquid containing from 33 to 40 per cent. of the gas and 10 to 20 per cent. of methyl-alcohol, its chief impurity. If the commercial solution—ordinarily known in the trade as "formalin"—is evaporated or concentrated above 40 per cent., paraformaldehyde results; and when this is dried *in vacuo* over sulphuric acid a third body—trioxymethylene—is produced, consisting of three molecules of CH_2O. This is a white powder, almost soluble in water or alcohol, and giving off a strong odor of formaldehyde. The solid polymers of formaldehyde, when heated, are again reduced to the gaseous condition; ignited, they finally take fire and burn with a blue flame, leaving but little ash. When burned they have no germicidal properties.

Formaldehyde has an active affinity for many organic substances, and forms with some of them definite chemical combinations. It combines readily with ammonia to produce a compound called hexamethylene tetramine, which possesses neither the odor nor the antiseptic properties of formaldehyde. This action is made use of in neutralizing the odor of formaldehyde when it is desired to dispel it rapidly after disinfection. Formaldehyde also forms combinations with certain aniline colors—viz., fuchsin and safranin—the shades of which are thereby changed or intensified. These dyes are tests for aldehydes. These are the only colors, however, which are thus affected, and as they are seldom used in dyeing, owing to their liability to fade, this effect is of little practical significance. The most delicate fabrics of silk, wool, cotton, fur, leather, etc., are unaffected in

texture or color by formaldehyde. Iron and steel are attacked, after long exposure, by the gas in combination with watery vapor; but copper, brass, nickel, zinc, silver, and gilt work are not at all acted upon. Formaldehyde unites with nitrogenous products of decay—fermentation or decomposition—forming true chemical compounds, which are odorless and sterile. It is thus a true deodorizer in that it does not replace one odor by another more powerful, but forms new chemical compounds which are odorless. Formaldehyde has a peculiar action upon albumin, which it transforms into an insoluble and indecomposable substance. It renders gelatin insoluble in boiling water and most acids and alkalies. It is from this property of combining chemically with the albuminoids forming the protoplasm of bacteria that formaldehyde is supposed to derive its bactericidal powers. Formaldehyde is an excellent preservative of organic products. It has been proposed to make use of this action for the preservation of meat, milk, and other food products; but, according to Trillat and other investigators, formaldehyde renders these substances indigestible and unfit for food. It has been successfully employed as a preservative of pathologic and histologic specimens.

There are no exact experiments recorded of the physiologic action of formaldehyde on the human subject when taken internally. A 1 per cent. solution has been taken in considerable quantity without serious results; and trioxymethylene has been given in doses up to 90 grains as an intestinal antiseptic. The vapors of formaldehyde are extremely irritating to the mucous membrane of the eyes, nose, and mouth, causing profuse lacrimation, coryza, and flow of saliva. Aronson reports that in many of his experiments rabbits and guinea-pigs allowed to remain for twelve and twenty-four hours in rooms which were being disinfected with formaldehyde gas were found to be perfectly well when the rooms were opened. On autopsy the animals showed no injurious effects of the gas. Others have noticed that animals, such as dogs and cats, which have accidently been confined for any length of time in rooms undergoing formaldehyde disinfection occasionally died from the effects of the gas. Many observers, however, have reported that insects, such as roaches, flies, and bedbugs, are not, as a rule, affected. The result of these observations would seem to indicate that although formaldehyde is comparatively non-toxic to the higher forms of animal life, nevertheless a certain degree of caution should be observed in the use of this agent. It is important to remember that formaldehyde is practically inert as an insecticide except in extremely great concentrations.

The researches of Pottevin and Trillat have shown that the germicidal power of the gas depends not only upon its concentration, but also upon the temperature and the condition of the objects to be sterilized. As with other gaseous disinfectants—viz., sulphur dioxide and chlorine—it has been found that the action is more rapid and complete at high temperatures—*i. e.*, at 35° to 45° C. (95° to 120° F.)—and when the test objects are moist than at lower temperatures and when

the objects are dry. Still, it has been repeatedly demonstrated by actual experiment in rooms that it is possible to disinfect the surface of apartments and articles contained in them, under the conditions of temperature and moisture ordinarily existing in rooms even in winter, by an exposure of a few hours to a saturated atmosphere of formaldehyde gas. The results of numerous experiments have shown that in the air 2.5 per cent. by volume of the aqueous solution, or 1 per cent. by volume of the gas, are sufficient to destroy fresh virulent cultures of the common non-spore-bearing pathogenic bacteria in a few minutes.

Stahl has shown that bandages and iodoform gauze can be kept well sterilized by placing in the jars containing them pieces of a preparation of paraformaldehyde in tablet form containing 50 per cent. of formaldehyde. The same experimenter has also succeeded in making carpets and articles of clothing germ-free by spraying them with 0.5 to 2 per cent. solution of formaldehyde for fifteen to twenty minutes without the color of the fabrics being in any way affected. The investigations of Trillat, Aronson, Pottevin, and others have shown that a concentration of 1/10000 of the aqueous solution (40 per cent.), equal to 1/25000 of pure formaldehyde, was safe and sufficiently powerful to retard bacterial growth.

A 2 per cent. watery solution of formalin destroys the vegetative forms of bacteria within five to thirty minutes. In our experiments formalin has upon the vegetative forms about one-half the strength of pure carbolic acid.

Chloroform ($CHCl_3$).—This substance, even in pure form, does not destroy spores, although it kills bacteria in vegetative form, even in 1 per cent. solution. Chloroform is used practically as an antiseptic in antitoxic sera and in blood serum for culture purposes. The chloroform is expelled from the serum by heating it to 55° C.

Iodoform (CHI_3).—This substance has but very little destructive action upon bacteria; indeed, upon most varieties it has no appreciable effect whatever. When mixed with putrefying matter, wound discharges, etc., the iodoform is reduced into soluble iodine compounds, which act partly destructively upon the bacteria and partly by uniting with the poisons already produced.

Carbolic Acid (C_6H_5OH).—Pure phenol crystallizes in long, colorless crystals. In contact with air it deliquesces. It has a penetrating odor, a burning taste, and is a corrosive poison. It is soluble at ordinary temperatures in about 15 parts of water. Carbolic acid dissolves in water with some difficulty and should be therefore thoroughly mixed. It is not destructive to fabrics, colors, metals, or wood, and does not combine as actively with albuminous matters as bichloride of mercury. It is therefore more suitable for the disinfection of fæces, etc. A solution having 1 part to 1000 inhibits the growth of bacteria; 1 part to 400 kills the less resistant bacteria, and 1 part to 100 kills the remainder. A 5 per cent. solution kills the less resistant spores within a few hours and the more resistant in from one day to four weeks. A slight increase in temperature aids the destructive action;

thus, even at 37.5° spores are killed in three hours. A 3 per cent. solution kills streptococci, staphylococci, anthrax bacilli, etc., within one minute. Carbolic acid loses much of its value when in solution in alcohol or ether. An addition of 0.5 HCl aids its activity. Carbolic acid is so permanent and so comparatively little influenced by albumin that it is rightly widely used in practical disinfection even in places of more powerful substances.

Cresol.—Cresol [$C_6H_4(CH_3)OH$] is the chief ingredient of the so-called "crude carbolic acid." This is almost insoluble in water, and therefore of restricted value. Many methods are used for bringing it into solution so as to make use of its powerful disinfecting properties. With equal parts of crude sulphuric acid it is a powerful disinfectant, but it is, of course, strongly corrosive. An alkaline emulsion of the cresols and other products contained in "crude" carbolic acid with soap is called creolin. It is used in 1 to 5 per cent. emulsions. It is fully as powerful as pure carbolic acid. Lysol is similar to creolin, except that it has more of the cresols and less of the other products. It and creolin are of about the same value.

Tricresol.—Tricresol is a refined mixture of the three cresols (metacresol, paracresol, and orthocresol). It is soluble in water to the extent of 2.5 per cent. and its disinfecting power is about three times as great as that of carbolic acid.

Creolin.—Creolin contains 10 per cent. of cresols held in solution by soap.

Lysol.—Lysol contains about 50 per cent. of cresols. It mixes with water in all dilutions.

Oil of turpentine, 1:200, prevents the growth of bacteria.

Camphor has very slight antiseptic action.

Creosote in 1:200 kills many bacteria in ten minutes; 1:100 failed to kill tubercle bacilli in twelve hours.

Essential oils: Cardéac and Meumir found that the essences of cinnamon, cloves, thyme, and others killed typhoid bacilli within one hour. Sandalwood required twelve hours.

Thymol and eucalyptol have about one-fourth the strength of carbolic acid (Behring).

Oil of peppermint in 1:100 solution prevents the growth of bacteria.

TABLES OF ANTISEPTIC VALUES.[1]

Alum	1 : 222	Mercuric chloride	1 : 14,300
Aluminum acetate	1 : 6000	Mercuric iodide	1 : 40,000
Ammonium chloride	1 : 9	Potassium bromide	1 : 10
Boric acid	1 : 143	Potassium iodide	1 : 10
Calcium chloride	1 : 25	Potassium permanganate	1 : 300
Calcium hypochlorite	1 : 1000	Pure formaldehyde	1 : 25,000
Carbolic acid	1 : 333	Quinine sulphate	1 : 800
Chloral hydrate	1 : 107	Silver nitrate	1 : 12,500
Cupric sulphate	1 : 2000	Sodium borate	1 : 14
Ferrous sulphate	1 : 200	Sodium chloride	1 : 6
Formaldehyde (40%)	1 : 10,000	Zinc chloride	1 : 500
Hydrogen peroxide	1 : 20,000	Zinc sulphate	1 : 20

[1] These figures are approximately correct, and represent the percentage of disinfectant required to be added to a fluid containing considerable organic material, in order permanently to prevent any bacterial growth. Solutions of half the given strength will inhibit the growth of most bacteria and prevent the growth of many varieties.

CHAPTER IX.

PRACTICAL DISINFECTION AND STERILIZATION (HOUSE, PERSON, INSTRUMENTS, AND FOOD)—STERILIZATION OF MILK FOR FEEDING INFANTS.

DISINFECTANTS AND METHODS OF DISINFECTION EMPLOYED IN THE HOUSE AND SICK-ROOM.

Disinfection and Disinfectants.—Sunlight, pure air, and cleanliness are always very important agents in maintaining health and in protecting the body against many forms of illness. When, however, it becomes necessary to guard against such special dangers as accumulated filth or contagious diseases, disinfection and general cleaning up are essential. In order that disinfection shall afford complete protection it must be thorough; and perfect cleanliness is better, even in the presence of contagious disease, than filth with poor disinfection.

Since all forms of fermentation, decomposition, and putrefaction, as well as the infectious and contagious diseases, are caused by microorganisms, it is the object of disinfection to kill these. Decomposition and putrefaction should at all times be prevented by the immediate destruction or removal from the neighborhood of the dwelling of all useless putrescible substances. In order that as few articles as possible shall be exposed to the germs causing the contagious diseases, and thus become carriers of infection, it is important when conditions allow of it that all articles not necessary for immediate use in the care of the sick person, especially upholstered furniture, carpets, and curtains, should be removed from the room before placing the sick person in it.

Agents for Cleansing and Disinfection.—Too much emphasis cannot be placed upon the importance of cleanliness, both as regards the person and the dwelling, in preserving health and protecting the body from all kinds of infectious disease. Sunlight and fresh air should be freely admitted through open windows, and personal cleanliness should be attained by frequently washing the hands and body, disinfecting linen fabrics infected by expectoration, bowel discharges, etc.

Cleanliness in dwellings, and in all places where men go, may, under ordinary circumstances, be well maintained by the use of the two following solutions:

1. **Soapsuds Solution.**—For simple cleansing, or for cleansing after the method of disinfection by chemicals described below, one ounce of common soda should be added to twelve quarts of hot soapsuds (soft soap and water).

2. **Strong Soda Solution.**—This, which is a stronger and more effective cleansing solution and also a fairly efficient disinfectant, is made by

dissolving one-half pound of common soda in three gallons of hot water. The solution thus obtained should be applied by scrubbing with a hard brush.

When it becomes necessary to arrest putrefaction or to prevent the spread of contagious diseases by surely killing the living germs which cause them, more powerful agents must be employed than those required for simple cleanliness, and these are commonly called disinfectants. The following are some of the most reliable ones:

3. **Heat.**—Complete destruction by fire is an absolutely safe method of disposing of infected articles of small value, but continued high temperatures not as great as that of fire will destroy all forms of life; thus, boiling or steaming in closed vessels for one-half hour will absolutely destroy all disease germs.

4. **Carbolic Acid Solution.**—Dissolve six ounces of carbolic acid in one gallon of hot water (200 grams in 4000 c.c.). This makes approximately a 5 per cent. solution of carbolic acid, which, for many purposes, may be diluted with an equal quantity of water. The commercial "soluble crude carbolic acid" which is cheaper and twice as effective as the carbolic acid, can be used for privies and drains.[1] It makes a white emulsion on account of its not entering readily into solution. Care must be taken that the pure acid does not come in contact with the skin.

5. **Bichloride Solution** (bichloride of mercury or corrosive sublimate).—Dissolve sixty grains of pulverized corrosive sublimate and two tablespoonfuls of common salt in one gallon of hot water. This solution, which is approximately 1:1000, must be kept in glass, earthen, or wooden vessels (not in metal vessels). For safety it is well to color the solution.

The carbolic and bichloride solutions are very poisonous when taken by the mouth, but are harmless when used externally.

6. **Milk of Lime.**—This mixture is made by adding one quart of dry, freshly slaked lime to four or five quarts of water. (Lime is slaked by pouring a small quantity of water on a lump of quicklime. The lime becomes hot, crumbles, and as the slaking is completed a white powder results. The powder is used to make milk of lime.) Air-slaked lime (the carbonate) has no value as a disinfectant.

7. **Dry Chlorinated Lime, "Chloride of Lime."**—This must be fresh and kept in closed vessels or packages. It should have the strong, pungent odor of chlorine.

8. **Formalin.**—Add 1 part of formalin to 10 of water. This equals in value the 5 per cent. carbolic acid solution.

9. **Creolin, Tricresol, and Lysol.**—The first is of about the same value as pure carbolic acid, the latter two about three times as powerful.

The proprietary disinfectants, which are so often widely advertised

[1] The cost of the pure carbolic acid solution is much greater than that of most of the other solutions, but except for the disinfection of the skin, which in some persons it irritates, and of woodwork, it is generally much to be preferred by those not thoroughly familiar with disinfectants, as it does not deteriorate, and is rather more uniform in its action than some of the other disinfectants.

and whose composition is kept secret, are relatively expensive and often unreliable and inefficient. It is important to remember that substances which destroy or disguise bad odors are not necessarily disinfectants, and that there are very few disinfectants that are not poisonous when taken internally. Their value should be stated in the circular in comparison with pure carbolic acid, so that their strength may be known.

Methods of Disinfection in Infectious and Contagious Diseases.—The diseases to be commonly guarded against, outside of surgery, by disinfection are scarlet fever, measles, diphtheria, tuberculosis, smallpox, typhoid and typhus fever, bubonic plague, and cholera.

1. **Hands and Person.**—Dilute the 5 per cent. carbolic solution with an equal amount of water or use the 1:1000 bichloride solution without dilution. Hands soiled in caring for persons suffering from contagious diseases, or soiled portions of the patient's body, should be immediately and thoroughly washed with one of these solutions and then washed with soap and water, and finally immersed again in the solutions. The nails should always be kept perfectly clean. Before eating, the hands should be first washed in one of the above solutions, and then thoroughly scrubbed with soap and water by means of a brush.

2. **Soiled clothing, towels, napkins, bedding, etc.**, should be immediately immersed in the carbolic solution, in the sick-room, and soaked for one or more hours. They should then be wrung out and boiled in the soapsuds solution for twenty minutes. Articles such as beds, woollen clothing, etc., which cannot be washed, should at the end of the disease be referred to the Health Department, if such is within reach, for disinfection or destruction; or if there is no public disinfection, these goods should be thoroughly exposed to formaldehyde gas, as noted later.

3. **Food and Drink.**—Food thoroughly cooked and drinks that have been boiled are free from disease germs. Food and drinks, after cooking or boiling, if not immediately used, should be placed when cool in clean dishes or vessels and covered. In the presence of an epidemic of cholera or typhoid fever, milk and water used for drinking, cooking, washing dishes, etc., should be boiled before using, and all persons should avoid eating uncooked fruit and fresh vegetables. Instead of boiling, milk may be heated to 80° C. for twenty minutes.

4. **Discharges of all kinds from the mouth, nose, bladder, and bowels** of patients suffering from contagious diseases should be received into glass or earthen vessels containing the carbolic solution, or milk of lime, or they should be removed on pieces of cloth, which are immediately immersed in one of these solutions or destroyed by fire. Special care should be observed to disinfect at once the vomited matter and the intestinal discharges from cholera patients. In typhoid fever the urine and the intestinal discharges, and in diphtheria, measles, and scarlet fever the discharges from the throat and nose all carry infection and should be treated in the same manner. The volume of the solu-

tion used to disinfect discharges should be at least twice as great as that of the discharge, and should completely mix with it and cover it. After standing for an hour or more the disinfecting solution with the discharges may be thrown into the water-closet. Cloths, towels, napkins, bedding, or clothing soiled by the discharges must be at once placed in the carbolic solution, and the hands of the attendants disinfected, as described above. In convalescence from measles and scarlet fever the scales from the skin are also carriers of infection. To prevent the dissemination of disease by means of these scales the skin should be carefully washed daily in warm soap and water. After use the soapsuds should be disinfected and thrown into the water-closet.

Masses of fæces are extremely difficult to disinfect except on the surface, for it takes disinfectants such as the carbolic acid solution some twelve hours to penetrate to their interior. If fæcal masses are to be thrown into places where the disinfectant solution covering them will be washed off, it will be necessary to be certain that the disinfectant has previously penetrated to all portions and destroyed the disease germs. This can be brought about by stirring them with the disinfectant and allowing the mixture to stand for one hour, or by washing them into a pot holding soda solution which is already at the boiling temperature, or later will be brought to one.

5. **Sputum from Consumptive Patients.**—The importance of the proper disinfection of the sputum from consumptive patients is still underestimated. Consumption is an infectious disease, and is always the result of transmission from the sick to the healthy or from animals to man. The sputum contains the germs which cause the disease, and in a large proportion of cases is the source of infection. After being discharged, unless properly disposed of, it may become dry and pulverized and float in the air as dust. This dust contains the germs, and is a common cause of the disease, through inhalation. In all cases, therefore, the sputum should be disinfected when discharged. It should be received in covered cups containing the carbolic or milk-of-lime solution. Handkerchiefs soiled by it should be soaked in the carbolic solution and then boiled. Dust from the walls, mouldings, pictures, etc., in rooms that have been occupied by consumptive patients, where the rules of cleanliness have not been carried out, contain the germs and will produce tuberculosis in animals when used for their inoculation; therefore, rooms should be thoroughly disinfected before they are again occupied. If the sputum of all consumptive patients were destroyed at once when discharged a large proportion of the cases of the disease would be prevented.

6. **Closets, Kitchen and Hallway Sinks, etc.**—The closet should never be used for infected discharges until they have been thoroughly disinfected; if done, one quart of carbolic solution or of 5 per cent. solution of formalin should be poured into the pan (after it is emptied) and allowed to remain there. Sinks should be flushed at least once daily.

7. **Dishes, knives, forks, spoons, etc.**, used by a patient should, as a rule, be kept for his exclusive use and not removed from the room.

They should be washed first in the carbolic solution, then in boiling hot soapsuds, and finally rinsed in hot water. These washing fluids should afterward be thrown into the water-closet. The remains of the patient's meals may be burned or thrown into a vessel containing the carbolic solution or milk of lime, and allowed to stand for one hour before being thrown away.

8. **Rooms and Their Contents.**—Rooms which have been occupied by persons suffering from contagious disease should not be again occupied until they have been thoroughly disinfected. It is true that when the patient is freed from isolation most of the disease germs have already died, but a few may have survived. The danger from infection is much greater when cases are removed during the acute illness. For disinfecting rooms either careful fumigation with formaldehyde gas or sulphur should be employed, or this combined with the following procedure: Carpets, curtains, and upholstered furniture which have been soiled by discharges, or which have been exposed to infection in the room during the illness, will be removed for disinfection to chambers where they can be exposed to formaldehyde gas and moderate warmth for twelve to twenty-four hours, or to steam. Some carpets, such as many Wiltons, are discolored by moist steam. These must be put in the formaldehyde chamber. Woodwork, floors, and plain furniture will be thoroughly washed with the soapsuds and bichloride solutions. After disinfection is finished it is well to remove the dried bichloride of mercury from the walls.

9. **Rags, cloths, and articles of small value,** which have been soiled by discharges or infected in other ways, should be boiled or burned.

10. **In case of death** the body should be completely wrapped in several thicknesses of cloth wrung out of the carbolic or bichloride solution, and when possible placed in an hermetically sealed coffin.

It is important to remember that *an abundance of fresh air, sunlight, and absolute cleanliness* not only helps protect the attendants from infection and aids in the recovery of the sick, but directly destroys the bacteria which cause disease.

Methods of Cleanliness and Disinfection to Prevent the Occurrence of Illness.—1. **Water-closet bowls and all receptacles for human excrement** should be kept perfectly clean by frequent flushing with a large quantity of water, and as often as necessary disinfected with the carbolic, bichloride, or other efficient solutions. The woodwork around and beneath them should be frequently scrubbed with the hot soapsuds solution.

2. **Sinks and the woodwork around and the floor beneath them** should be frequently and thoroughly scrubbed with the hot soapsuds solution.

3. **School Sinks.**—School sinks should be thoroughly flushed with a large quantity of water at least twice daily, and should be carefully cleaned twice a week or oftener by scrubbing. Several quarts of the crude carbolic solution should be frequently thrown in the sink after it has been flushed.

4. **Cesspools and Privy Vaults.**—An adundance of milk of lime or chloride of lime should be thrown into these daily, and their contents should be frequently removed.

5. **Refrigerators and the surfaces around and beneath them, dumb-waiters, etc.,** may be cleaned by scrubbing them with the hot soapsuds solution.

6. **Traps.**—All traps should be flushed daily with an abundance of water. If at any time they become foul they may be cleaned by pouring considerable quantities of the hot strong soda solution into them, followed by the carbolic or formalin solution.

7. **The woodwork in school-houses** should be scrubbed daily with hot soapsuds. This refers to floors, doors, door-handles, and all woodwork touched by the scholars' hands.

8. **Spittoons in all public places** should be emptied daily and washed with the hot soapsuds solution, after which a small quantity of the carbolic solution or milk of lime should be put in the vessel to receive the expectoration.

9. **Cars, Ferry-boats, and Public Conveyances.**—The floors, door-handles, railings, and all parts touched by the hands of passengers should be washed frequently with the hot soapsuds solution. Slat-mats from cars, etc., should be cleaned by scrubbing with a stiff brush in the hot soapsuds solution.

Telephone receiver mouth-pieces should also be frequently cleansed.

Use of Bromine Solution as a Deodorant.—*Slaughter-houses, butchers' ice-boxes and wagons, trenches, excavations, stable floors, manure-vaults, dead animals, offal, offal docks, etc.,* may be deodorized by a weak solution of bromine, which is a valuable agent for this purpose. The bromine solution, however, is only temporary in its action, and must be used repeatedly. It should be applied by sprinkling. Although somewhat corrosive in its action on metals, it is otherwise harmless.

The solution of bromine must be prepared with great care, as the pure bromine from which it is made is dangerous. It is very caustic when brought in contact with the skin; it is volatile and its fumes are very irritating when inhaled. To prepare the solution an ounce bottle of liquid bromine is dropped into three gallons of water, and broken under the water and thoroughly stirred.

The Practical Employment of Formaldehyde Gas in the Surface Disinfection of Rooms and the Disinfection of Goods which would be Injured by Heat.—Formaldehyde gas has come into such general use, and is for many purposes so valuable, that the description of methods employed to generate and use it will be given in detail.

If we consider now the practical application of formaldehyde gas for purposes of disinfection we find that its destructive action on microörganisms depends upon a number of factors, the chief of which are its concentration in the surrounding atmosphere, the length of the contact, the existing temperature, the accompanying moisture, and the nature of the organism.

The necessary concentration of gas in the surrounding atmosphere to kill the microörganisms varies with each species, for some resist chemical agents much more than others, and also with the freedom of access of the gas to the bacteria, for if they are under cover or within fabrics a greater amount of gas must be generated than if they are freely exposed.

For purely surface disinfection, when the less resistant bacteria or other microörganisms are to be destroyed, there will be required, according to the method used, ten to twelve ounces of formalin of full strength, or its equivalent, to 1000 cubic feet of air space.

For the destruction of the more resistant but non-spore-bearing forms, such as typhoid fever or tubercle bacilli, at least twelve ounces of formalin should be used. The gas penetrates through fabrics with difficulty, and to pass through heavy goods the concentration of the gas must be doubled and moderate heat added (45° C. or above).

Value of Moisture.—At first it was thought that formaldehyde gas acted more effectually in a dry atmosphere, but further investigation has proved that, although it does destroy bacteria with the amount of moisture usually present in the air, and contained in their own substance, it acts much more powerfully and certainly when additional moisture is present, and best when present up to the point of saturation. The actual spraying with water of walls and goods to be disinfected is even more efficacious.

A fairly high temperature—but one still below that which would injure delicate fabrics—increases not only the activity of formaldehyde gas, but also its penetrative power, and for heavy goods it is essential. The production of a partial vacuum in the chambers before the introduction of the formaldehyde gas still further assists its penetration.

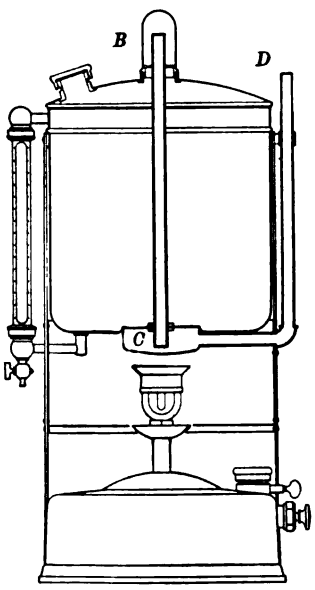
The length of exposure necessary for complete disinfection depends upon the nature of the disease for which it is carried out—the penetration required, the concentration of the gas used, the amount of moisture in the air, the temperature of the air, and the size and shape of the room. For surface disinfection in rooms, when as much as twelve ounces of formalin are used for each 1000 cubic feet, five hours' exposure is amply sufficient, most bacteria being killed within the first thirty minutes. For the destruction of microörganisms protected by even a layer of thin covering, double the formalin and double the time of exposure should be allowed, and even then the killing of many species of non-spore-bearing bacteria cannot be counted upon in ordinary rooms. When absolutely complete disinfection is demanded, where penetration of gas is required, the goods must be placed in chambers where moderate heat can be added and all leakage of gas prevented.

Various forms of apparatus can be properly employed to liberate formaldehyde gas for purposes of disinfection. There are two essentials to any good method—namely, that the formaldehyde gas is given off quickly, and that there is no great loss by deterioration of the formalin.

Wood Alcohol.—A number of lamps have been devised, all very much on the same principle, though varying somewhat in mechanical construction, which bring about the incomplete oxidation of methyl-alcohol by passing the vapors mixed with air over the incandescent metal. Although disinfection can be carried out by the best of these lamps, in our experience none of them up to the present time are satisfactory or economical. They may be very useful as deodorizers in the sick-room or other places.

The same principle is used efficiently in another form. The vapor of wood alcohol is passed over the surfaces of asbestos containing particles of finely divided platinum. This apparatus has given very good results, and for a given amount of disinfection leaves less odor of formaldehyde gas in the room than any other. The apparatus is, however, bulky and expensive.

FIG. 63



Formaldehyde apparatus.

Formalin by Boiling and Passing the Vapor through a Superheated Coil or Chamber.—This system consists in heating the ordinary commercial formalin to a high temperature in an incandescent copper coil or chamber, and allowing the vapors to pass off freely. It is claimed for this method that the degree of heat necessary to break up the polymerized products formed is supplied, and thus a loss of formaldehyde is prevented. A further action of the intense heat in the copper tube on the solution is partially to convert the methyl-alcohol contained in commercial formalin into formaldehyde gas by partial oxidation, thereby utilizing a part of the methyl-alcohol and increasing the amount of formaldehyde.

In operation the desired quantity of formalin is placed in the receiver and the receiver is closed. The lamp is lighted and the coil brought to a red heat. The valve is then opened and the solution contained in the receiver is allowed to pass down and into the coil in a fine stream. Upon coming in contact with the heated metal the formaldehyde solution is instantly decomposed, and the liberated gas is further purified as it progresses through the incandescent coil. The apparatus is liable to get out of order, in that the valve is apt to become clogged and so stop the flow of formalin until freed by a wire supplied for the purpose.

In the apparatus (Fig. 63) the formalin is first boiled in the large chamber and passes as vapor through the tube connecting *B* and *C*. In *C* it is superheated and passes out the tube *D* through a rubber tube into the room. In all forms of apparatus where formalin is used the large receiving chamber should be washed out from time to time with hot water, to remove any deposit there may be.

Trioxymethylene by Schering's System.—This system consists in heating the solid polymer of formaldehyde (trioxymethylene) in a lamp specially constructed for the purpose. The trioxymethylene is used in the form of compressed tablets or pastilles, as being more convenient for use. Each pastille contains the equivalent of 100 per cent. of formaldehyde gas, according to the manufacturers, and weighs 1 gram.

The mode of using the apparatus is very simple: The disinfector is placed upon a sheet of iron on the floor of the room to be disinfected. From 100 to 250 pastilles can be evaporated at a time in the apparatus. For the production of greater quantities of formaldehyde vapor several of these outfits may be used together. The lamp is filled with ordinary or wood alcohol, about twice as many cubic centimetres of the alcohol being employed as there are pastilles to be evaporated. The wicks should project but little above the necks of the burners, or the apparatus may get too hot and ignite the pastilles. The vessel is charged with formalin pastilles and the disinfector placed over the lighted spirit lamp. The lamp is then allowed to burn out in the closed room. One hundred pastilles are considered to be sufficient for the disinfection of 1000 cubic feet of space. Lately, a small steam boiler has been added to the apparatus, for the purpose of furnishing sufficient moisture with the gas. The results obtained by us in superficial disinfection, when from 150 to 200 pastilles have been used to each 1000 cubic feet, have been good. The great advantage of the method is in the small cost of the apparatus, $3.00, and the avoidance of the danger of deterioration, which is present to some extent in formalin. Smaller lamps are very useful for the deodorization of rooms.

From Pastilles Composed of a Top of Compressed Paraform and a Base of Prepared Charcoal.—This is a very neat but somewhat expensive method of liberating formaldehyde gas. Our results with it have been good.

Formalin to which Glycerin has been Added.—To the formalin is added 10 per cent. of glycerin, and the mixture is simply boiled in a suitable copper vessel, the steam and formaldehyde gas passing off by a tube. This is a very serviceable apparatus. When it is attempted to vaporize the formalin too rapidly part of it bubbles over in fluid form.

With 50 per cent. more of formalin than that used in the high temperature autoclave and heated tube or chamber methods, the results seem to be equally as good. The apparatus is very easy to use, and is not liable to get out of order.

Similar forms of apparatus are also employed, when instead of glycerin the formalin is mixed with an equal quantity of water. The water is for the purpose of giving additional moisture to the air, and, at the same time, like the glycerin, to prevent the change of formaldehyde into inert substances.

From Formalin in an Open Pan.—A very simple method, devised by Dr. R. J. Wilson, is to fill a tin pan with twelve ounces of formalin

for each 1000 cubic feet and put this on an upright sheet of tin, which is cut so as to allow of the entrance of air below and yet protect the formalin in the pan from the flame. For heating put under it a small tin can filled with asbestos packing which has been soaked with wood alcohol. A still simpler method is to pour on folded sheets sixteen ounces of formalin per 1000 cubic feet and then stretch them out over lines in a room and leave for ten hours. If the room is tightly sealed very fair surface disinfection will take place.

Lime and Permanganate Method of Generating Formaldehyde Gas.—Satisfactory results in disinfection have been obtained from the following combination of chemicals. Two ounces of a quick-slaking, coarsely granular lime (calcium oxide); 5 ounces of permanganate of potash; ½ gram oxalic acid; 5 ounces formaldehyde solution, 40 per cent. strength; and 2½ ounces of water. This is sufficient in quantity to disinfect 1000 cubic feet of space in five hours. It is used as follows: The lime and permanganate of potash are mixed together in a pan at least 10½ inches in diameter and 3½ to 4 inches in depth. Over this is poured the freshly prepared mixture of formaldehyde solution, oxalic acid, and water. A rapid evolution of gas takes place.

Another combination is lime 2.7 ounces; potassium permanganate, 5.5 ounces; formaldehyde solution, 7.4 ounces; water, 2.7 ounces. The technic is as follows: The lime and permanganate are mixed in a wide, deep pan as above, and the freshly prepared formaldehyde and water mixture is poured over it.

Permanganate of potash method. The following combination will also disinfect 1000 cubic feet of space in five hours: potassium permanganate, 10 ounces; formaldehyde solution, 40 per cent., 9 ounces; water, 4.5 ounces. The formaldehyde and water are mixed together and rapidly poured over the permanganate of potash. The reaction is immediate and violent. This mixture requires a deep, wide pan or a pail at least 18 inches deep. The addition of the water is believed to increase the liberation of the formaldehyde gas.

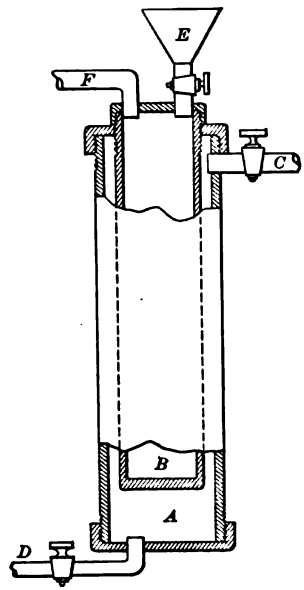
Lime Method of Generating Formaldehyde Gas.—To ten ounces of 40 per cent. formaldehyde solution slowly add one ounce of concentrated sulphuric acid; pour this solution on to two pounds of quicklime that had previously been cracked into small lumps and placed in a dairy pan not less than twelve inches in diameter. The liberation of a large amount of gas in a short time more than compensates for the loss by polymerization, and disinfection is effected by a quick union of the gas and organisms to be destroyed. Saturated solution of aluminum sulphate may be used instead of concentrated sulphuric acid.

Rapid Generation of Formaldehyde Gas for Large Chambers by the Method of Dr. R. J. Wilson.—The generator (Fig. 64) is made of ordinary iron steam pipe and can be manufactured in any pipe-cutting establishment in a very few hours. It consists of an outer steam jacket of six-inch pipe, two feet long, and capped at both ends. Through the upper cap there is a four-inch opening, with a thread, through which projects an inner chamber for formalin. This chamber con-

sists of a four-inch pipe, twenty-two inches long, capped at the upper end and welded or capped at the lower end. The upper end of this pipe is so threaded as to permit of its being screwed through the cap of the steam jacket before that cap is screwed on. The cap of the formalin chamber is fitted on the same thread that passes through the cap of the steam jacket. The in-take for steam is near the top of the steam jacket, through a half-inch pipe, and the steam is controlled by a globe valve. The outlet for steam or drip is through a half-inch pipe from the bottom cap of the chamber and is also controlled by a globe valve. The in-take for formalin is through the upper cap of the formalin chamber through a half-inch pipe controlled by a globe valve. The outlet for formaldehyde is a half-inch pipe through the upper cap of the formalin chamber.

This generator is cheap and efficient, but considerable care should be observed in operating it, as there is a tendency to throw out some formalin before the gas begins to be evolved. This is easily avoided by using care in the proper application of the heat. These generators have now been in use for eight years by the New York Health Department, and have given complete satisfaction.

FIG. 64



Wilson's Formaldehyde Generator.

A, steam chamber; *B*, formalin chamber; *C*, steam supply; *D*, drip; *E*, inlet for formalin; *F*, outlet for formaldehyde.

As a result of the investigations undertaken in the Department of Health laboratories on the use of formaldehyde as a disinfectant, and a consideration of the work of others, the conclusions reached by us may be summarized as follows:

1. GENERAL RULES FOR DISINFECTION OF INFECTED DWELLINGS.—Exposed surfaces of walls, carpets, hangings, etc., in rooms may be superficially disinfected by means of formaldehyde gas. All apertures in the rooms should be tightly closed and from ten to sixteen ounces of formalin or its equivalent used to generate the gas for each 1000 cubic feet. The time of exposure should be not less than four hours, and a suitable apparatus should be employed. The temperature of the apartment should be as high as possible, and certainly not below 50° F. With even lower temperature surface disinfection is possible, but larger amounts of formalin must be used. When generated very rapidly the formaldehyde gives much better results than when given off slowly.

Under these conditions spore-free bacteria and the contagion of the exanthemata are surely destroyed when freely exposed to the action of the gas. Spore-bearing bacteria are not thus generally destroyed; but these latter are of such rare occurrence in disease that in house

disinfection they may usually be disregarded, and, if present, special measures can be taken.

The penetrative power of formaldehyde gas in the ordinary room, at the usual temperature, even when used in double the strength necessary for surface disinfection, is extremely limited, not passing, as a rule, through more than one layer of cloth of medium thickness. Articles, therefore, such as bedding, carpets, upholstery, clothing, and the like should, when possible, be subjected to steam, hot air, or formaldehyde disinfection in special chambers constructed for the purpose. If not, they must be thoroughly exposed on all sides.

2. Disinfection of Bedding, Carpets, Upholstery, Etc.—Bedding, carpets, clothing, etc., which would be injured by steam, may be disinfected by means of formaldehyde gas in an ordinary steam disinfecting chamber, the latter to be provided with a heating and if possible a vacuum apparatus and special apparatus for generating the gas. Where penetration through heavy articles is required the gas should be used in the proportion of not less than the amount derived from thirty ounces of formalin for each 1000 cubic feet, the time of exposure to be not less than eight hours and the temperature of the chamber not below 100° F.

In order to insure complete sterilization of the articles they should be so placed as to allow of a free circulation of the gas around them —that is, in the case of bedding, clothing, etc., these should either be spread out on perforated wire shelves or loosely suspended in the chamber. The aid of a partial vacuum facilitates the operation. Upholstered furniture and articles requiring much space should be placed in a large chamber, or, better, in a room which can be heated to the required temperature.

The most delicate fabrics, furs, leather, and other articles, which are injured by steam, hot air at 230° F., or other disinfectants, are unaffected by formaldehyde.

3. Disinfection of Books.—Books may be satisfactorily disinfected by means of formaldehyde gas in a special room, or in the ordinary steam chamber, as above described, and under the same condition of volume of gas, temperature, and time of exposure. The books should be arranged to stand as widely open as possible upon perforated wire shelves, set about one or one and a half feet apart in the chamber. A chamber having a capacity of 200 to 250 cubic feet would thus afford accommodation for about one hundred books at a time.

Books, with the exception of their surfaces, cannot be satisfactorily disinfected by formaldehyde gas in the bookcases of houses or libraries, or anywhere except in special chambers constructed for the purpose, because the conditions required for their thorough disinfection cannot otherwise be complied with.

The bindings, illustrations, and print of books are in no way affected by the action of formaldehyde gas.

4. Disinfection of Carriages, Etc.—Carriages, ambulances,

cars, etc., can easily be disinfected by having built a small, tight building, in which they are enclosed and surrounded with formaldehyde gas. Such a building is used for disinfecting ambulances in New York City. With the apparatus there employed a large amount of formalin is rapidly vaporized, and superficial disinfection is completed in sixty minutes.

5. METHOD FOR TESTING EFFICACY OF ROOM DISINFECTION.—The following method modified by Dr. Schroeder, working in the Research Laboratory, is now in use in the Department of Health.

The main points of the system are as follows:

No. 36 cotton is cut into inch lengths, placed in a Petri dish, and covered with a forty-eight-hour broth culture of pyocyaneus.

They are left for two or three minutes or until they are thoroughly saturated, then removed to filter-paper in another covered Petri dish and left to dry. When dry, they are placed in tissue-paper envelopes, which are stamped with all necessary data. Each envelope is dated and sealed and sent to the disinfector who places it in the room which is to be disinfected.

The driver who calls for the bedding takes up the tests, placing them in a manilla envelope and entering them upon his card. The envelopes are then returned to the laboratory where the tests and receipt card are compared and any discrepancy noted.

The test envelopes are then stamped with date of receipt, and the threads are removed and placed in a modified Ayer's medium, which is a synthetic medium and consists of the following:

Asparagin	4
Neutral NaPhos	2
Sodium lact	6
Sodium chlor	5
Water	1000

Add enough NaOH to render the medium alkaline to litmus. This culture medium may be depended upon to give bright green color reaction in twenty-four to forty-eight hours.

The tubes are incubated for forty-eight hours and the color reaction noted and entered upon test envelope.

Upon completion of this process the envelopes are sorted upon a table marked off into alphabetical spaces. They are then entered upon the disinfector's card.

At the end of the week a bacteriologist's report is compiled which shows at a glance the work of each disinfector, the number of cases of each disease for which disinfection was performed, the number of successful disinfections, the number of tests lost, etc.

6. ADVANTAGES OF FORMALDEHYDE GAS OVER SULPHUR DIOXIDE FOR DISINFECTION OF DWELLINGS.—Formaldehyde gas is superior to sulphur dioxide as a disinfectant for dwellings: first, because it is more efficient in its action; second, because it is less injurious in its

effects on household goods; third, because when necessary it can easily be supplied from a generator placed outside of the room and watched by an attendant, thus avoiding in some cases danger of fire.

Apart from the cost of the apparatus and the greater time involved, formaldehyde gas, generated from commercial formalin, is not much more expensive than sulphur dioxide—viz., twelve to twenty cents per 1000 cubic feet against ten cents with sulphur. Therefore, we believe that formaldehyde gas is the best disinfectant at present known for the surface disinfection of infected dwellings. For heavy goods it is far inferior in penetrative power to steam; but for the disinfection of fine wearing apparel, furs, leather, upholstery, books, and the like, which are injured by great heat, it is, when properly employed, better adapted than any other disinfectant now in use.

Sulphur Dioxide in House Disinfection.—Four pounds of sulphur should be burned for every 1000 cubic feet. The sulphur should be broken into small pieces and put into a pan sufficiently large not to allow the melted sulphur to overflow. This pan is placed in a much larger pan holding a little water. The cracks of the room should be carefully pasted up and the door, after closing, also sealed. Upon the broken sulphur is poured three to four ounces of alcohol and the whole lighted by a match. The alcohol is not only for the purpose of aiding the sulphur to ignite, but also to add moisture to the air. An exposure of eight to twelve hours should be given.

Sulphur fumigation carried out as above indicated is not as efficient as formaldehyde fumigation, but suffices for surface disinfection for diphtheria and the exanthemata. All heavy goods should be removed for steam disinfection if there is any possibility of the infection having penetrated beneath their surface. If there is no place for steam disinfection their surfaces should be thoroughly exposed to fumigation and then to the air and sunlight. In many cases when cleanliness has been observed, surface disinfection of halls, bedding, and furniture may be all that will be required.

There is always a very slight possibility of a deeper penetration of infection than that believed to have occurred; it is, therefore, better to be more thorough than is considered necessary rather than less.

Sulphur dioxide without the addition of moisture has, as already stated under the consideration of disinfectants, very little germicidal value upon dry bacteria.

Public Steam Disinfecting Chambers.—These should be of sufficient size to receive all necessary goods, and may be either cylindrical or rectangular in shape, and are provided with steam-tight doors opening at either end, so that the goods put in at one door may be removed at the other. When large the doors are handled by convenient cranes and drawn tight by drop-forged steel eye-bolts swinging in and out of slots in the door frames. The chambers should be able to withstand a steam pressure of at least one-half an atmosphere, and should be constructed with an inside jacket, either in the form of an inner and outer shell or of a coil of pipes. This jacket is filled with

steam during the entire operation, and is so used as to bring the goods in the disinfecting chamber up to the neighborhood of 220° F. before allowing the steam to pass in. This heats the goods, so that the steam does not condense on coming in contact with them. It is an advantage to displace the air in the chamber before throwing in the steam, as hot air has far less germicidal value than steam of the same temperature. To do this, a vacuum pump is attached to the piping, whereby a vacuum of fifteen inches can be obtained in the chamber. The steam should be thrown into the chamber in large amount, both above and below the goods, and the excess should escape through an opening in the bottom of the chamber, so as more readily to carry off with it any air still remaining. The live steam in the chamber should be under a pressure of two to three pounds so as to increase its action.

To disinfect the goods, we place them in the chamber, close tight the doors, and turn the steam into the jacket. After about ten minutes, when the goods have become heated, a vacuum of ten to fifteen inches is produced, and then the live steam is thrown in for twenty minutes. The steam is now turned off, a vacuum is again formed, and the chamber again superheated. The goods are now throughly disinfected and dry. In order to test the thoroughness of any disinfection, or any new chamber maximum, thermometers are placed, some free in the chamber and others surrounded by the heaviest goods. It will be found that, even under a pressure of three pounds, live steam will require ten minutes to penetrate heavy goods.

The Disinfection of Hands, Instruments, Ligatures, and Dressings for Surgical Operations.—Instruments.—All instruments, except knives, after having been thoroughly cleansed, are boiled for three minutes in a 1 per cent. solution of washing soda. Knives, after having been thoroughly cleansed, are washed in sterile alcohol and wiped with sterile gauze and then put into boiling soda solution for one minute. This will not injure their edges to any great extent.

Gauze.—Gauze is sterilized by moist heat either in an Arnold steam sterilizer for one hour or in an autoclave for thirty minutes. It is placed in a perforated cylinder or wrapped in clean towels before putting in the sterilizer, and only opened at the operation.

Iodoform gauze is best made by sprinkling sterile iodoform on plain gauze sterilized as described above.

Ligatures—Catgut.—Boil for one hour in alcohol under pressure at about 97° C. It is often put in sealed glass tubes, which are boiled under pressure. These remain indefinitely sterile. The alcohol does not injure the catgut. If desired, the catgut can be washed in ether and then soaked a short time in bichloride before heating in alcohol. Boeckman, of St. Paul, suggested wrapping the separate strands of catgut in paraffin paper and then heating for three hours at 140° C. This procedure prevents the drying out of the moisture and fat from the catgut, so that it remains unshrivelled and flexible after its exposure. Darling, of Boston, tested this method and found it satisfactory. Dry formaldehyde gas does not penetrate sufficiently, and is not

reliable. Silver wire, silk, silkworm gut, rubber tubing, and catheters are boiled the same as the instruments.

Hand Brushes.—These should be boiled in soda solution for ten minutes.

The Skin of the Patient.—It is impossible absolutely to sterilize the deeper portions of the skin, but sufficient bacteria can be removed to render infection rare. The skin is washed thoroughly with warm green soap solution, then with alcohol, and finally with 1:1000 bichloride. A compress wet with a 25 per cent. solution of green soap is now placed on, covered with rubber tissue, and left for three to twelve hours; and after its removal the skin is washed with ether, alcohol, and bichloride solution, and then covered with a gauze compress previously moistened with a 1:1 000 bichloride of mercury solution. At the operation the skin is again scrubbed with green soap solution followed by ether, alcohol, and then with the bichloride of mercury solution. In some places the bichloride compress is replaced one hour before the operation by a pad wet in 10 per cent. solution of formalin.

The Hands.—Fürbinger's method, slightly modified, is now much used, and gives good results. The hands are washed in hot soap and water for five minutes, using the nail brush. They are then soaked in 85 per cent. alcohol for one minute and scrubbed with a sterile brush. They are finally soaked in a 1:1000 bichloride of mercury solution for two minutes. The alcohol and bichloride of mercury are sometimes combined and used together. Another method which gives good results is as follows: Skin of operator is scrubbed for five minutes with green soap and brush, then washed in chlorinated lime and carbonate of soda in proportions to make a good lather; washed off in sterile water, and then scrubbed with brush in warm bichloride solution 1:1000.

Owing to the risk of leaving untouched bacteria under the nails and in cracks of the skin, sterilized rubber gloves are now being used more and more in operations. Some surgeons prefer sterilized cotton gloves frequently changed. The gloves can be sterilized by steam.

Mucous Membranes.—Here absolute sterilization cannot be achieved without serious injury to the tissues. Those of the mouth and throat are cleansed by a solution consisting of equal parts of peroxide of hydrogen and lime-water. In the nostrils it is better to employ the milder solutions, such as diluted Dobell's or Listerine. These are also used in the mouth instead of the peroxide. Wadsworth[1] urges the use of preparations containing about 30 per cent. of alcohol as being very efficient.

The vagina is swabbed out thoroughly with sterile warm soap and water, and then irrigated with a 2 per cent. carbolic acid or a 1:1000 bichloride of mercury solution.

Hypodermic and Other Syringes.—These when not boiled are sterilized by drawing up into them boiling water a number of times and then finally a 5 per cent. solution of carbolic acid, the acid after three

[1] Mouth Disinfection. Jour. Infect. Dis., 1906, page 779.

minutes to be washed out by boiling water. If cold water is used the carbolic solution should remain in the barrel for ten minutes. Great care should be taken to wash out all possible organic matter before using the carbolic acid or boiling to sterilize. Syringes made entirely of glass or of glass and asbestos can be boiled in soda solution.

The Sterilization of Milk.—Complete sterilization destroys all the germs in milk, and so prevents permanently fermentative changes. This requires boiling for fifteen to forty-five minutes on two or three consecutive days, according to the presence or absence of certain spores. By partial sterilization most of the germs which are not in the spore form may be destroyed, so that the milk will remain wholesome for at least twenty-four hours when kept under proper conditions.

Milk is best sterilized by heat, for nearly all chemicals, such as boric acid, salicylic acid, and formalin, are not only slightly deleterious themselves but also make the milk less digestible, and, therefore, less fit for food. Formalin is the least objectionable of the three. Milk may be sterilized at a high or low temperature—that is, at the boiling temperature—or at a lower degree of heat, obtained by modifying the steaming process.

Pasteurization.—Milk sterilized at as high a temperature as 100° C. is not altogether desirable for prolonged use for infants, as the high temperature causes certain changes in the milk which make it less suitable as a food for them. These changes are almost altogether avoided if a temperature below 80° C. is used. It is recommended, therefore, that the lowest temperature be used for partial sterilization which will keep the milk wholesome for twenty-four hours in the warmest weather and kill the tubercle, typhoid, and other non-spore-bearing bacilli. Raising the milk to a temperature of 60° C. for twenty minutes, 65° C. for fifteen, 70° for five, 75° for two, or 80° for one will accomplish this. Exposure for even one minute at 70° destroys 98 per cent. of the bacteria which are not in the spore form. Fully 99 per cent. of tubercle bacilli are destroyed. This subject is considered more fully in the chapter on milk. One of the many forms of apparatus is the following:

(*a*) A tin pail or pot, about ten inches deep by nine inches in diameter, provided with the ordinary tin cover which has been perforated with eight holes each an inch in diameter.

(*b*) A wire basket, with eight nursing bottles (as sold for this purpose in the shops).

(*c*) Rubber corks for bottles and a bristle brush for cleaning them.

Directions (Koplik).—Place the milk, pure or diluted (as the physician may direct), in the nursing bottles and place the latter in the wire basket. Put only sufficient milk for one nursing in each bottle. Do not cork the bottles at first.

Having previously poured about two inches of water in the tin pail or pot and brought it to the boiling point, lower the basket of nursing bottles slowly into the pot. Do not allow the bottles to touch the water or they will crack. Put on the perforated cover and let the

steaming continue for ten minutes; then remove the cover and firmly cork each bottle. After replacing the cover, allow the steaming to continue for fifteen minutes. The steam must be allowed to escape freely or the temperature will rise too high.

The process is now completed. Place the basket of bottles in a cool, dark place or in an ice-chest. The bottles must not be opened until just before the milk is to be used, and then it may be warmed by plunging the bottle in warm water. If properly prepared the milk will taste but little like boiled milk.

The temperature attained under the conditions stated above will not exceed in extreme cases 87° C. (188° F.).

A different but admirable method is the one devised by Dr. Freeman.[1] Here a pail is filled to a certain mark with water, and then placed on the stove until the water boils. It is then removed, and immediately a milk-holder, consisting of a series of zinc cylinders, is lowered with its milk bottles partially full of milk. The cover is again applied. The heat of the outside water raises the temperature of the milk in ten minutes to about 65° C. (150° F.), and holds it nearly at that point for some time. After twenty minutes the milk is removed, placed in cold water, and quickly cooled. The milk is kept in the ice-chest until used.

Milk should be pasteurized when it is as fresh as possible, and only sufficient milk for twenty-four hours should be pasteurized at one time. If after nursing the infant leaves some milk in the bottle this should be thrown away.

Care of the Bottles.—After nursing, the bottles should be filled with a strong solution of washing soda, allowed to stand twenty-four hours, and then carefully cleaned with a bristle (bottle) brush. The rubber corks and nipples after using should be boiled in strong soda solution for fifteen minutes and then rinsed and dried.

After sterilizing milk should never be put into unsterilized bottles, as this will spoil it.

[1] Agent for Pasteurizer, James Dougherty, 411 W. 59th St.

CHAPTER X.

THE RELATION OF BACTERIA TO DISEASE.

In preceding chapters we have considered the growth of bacteria for the most part in dead organic substances. Now we have to consider the growth of bacteria and the production of their poisons in the living host and the results of such development. While it is true that there is a great difference between living and dead matter, and that, therefore, the living animal cannot be looked upon as merely a quantity of special material to be used for food for bacterial growth, still, in a very real sense, we are warranted in considering the infected living body as a food mass more or less favorable for bacterial growth. The difference is that besides the chemical substances, temperature, and conditions inherent to the fluids of the living body and its tissues, microörganisms have also to reckon with the constant production of new substances by the living cells of the invaded organism, which may be antagonistic to them. In the production of lesions by microörganisms there are four main factors involved—viz., on the part of micoörganisms, the power to elaborate poison and the ability to multiply; on the part of the body the degree of sensitiveness to the poisons of the bacteria and the tendency to produce antitoxic or bactericidal substances. No known variety of bacterial cell has as a single organism the ability to produce enough poison to do appreciable injury in the body, nor is it probable that there is any variety which, if it multiplied in the body to the extent that some pathogenic bacteria are capable of, would not produce disease. As already mentioned, varieties of bacteria even under similar conditions differ enormously in the amount of poison which they produce and in their ability after gaining entrance to multiply in the body.

To understand the bacterial factor in the production of disease we must recognize that both the body invaded and the bacteria which invade are living organisms, and that the products of the cellular activity of the body act on the bacteria at the same time the bacterial products act upon the human cells. Just as there are different races and species of animals having dissimilar characteristics, there are different races and species among bacteria, and just as the descendants of one animal species under changing conditions gradually become diverse, so do the descendants of one bacterial species. In fact, the rapidity of the development of new generations of bacteria allow in them of much quicker changes under new conditions than are possible in the higher animals and plants. Considering these and other facts, we can readily understand how the different types of bacteria do not grow equally well in every variety of animal, and after discovering that there are variations in the bacterial properties of the blood from

day to day we are not surprised that they do not find the body of the same animal always equally suitable. The study of bacteria in the more simple and known conditions of artificial culture media has shown us how extremely sensitive many bacteria are to slight chemical, and other changes. We have also found that conditions which are favorable to multiplication may still be unfavorable for the production of poisons.

If we take specimens of diphtheria bacilli from three different cases of diphtheria, we sometimes find that on growing them for several days in suitable bouillon one culture will have produced poison in the fluid to such a degree that a single drop suffices to kill a large guinea-pig; the second, grown in a similar manner, will kill another animal of the same size with half a drop; while the third will kill with one-tenth of a drop. This illustrates the important fact that different varieties of the same bacillus have different toxin-producing powers under the same conditions.

Let us now cultivate these same strains in bouillon which is a little too acid or a little too alkaline for their maximum development, and we shall find that while all of them will grow, only one and probably that one which produced the most toxin under favorable conditions will continue to develop it, while the others will fail to produce any specific poison. This illustration makes clear one reason for the variation in severity among different cases in an epidemic, since the conditions in one throat may favor growth but not toxin production, while in another throat both are favored. The fact that growth of bacteria may occur in the body and yet but little poison be produced, and that, of the same species of bacteria, some varieties are capable of producing specific poisons under less favorable circumstances than others, is very important to remember.

The cultivation of the tetanus bacillus also furnishes some interesting facts which illustrate the complicated ways in which the growth of varieties of bacteria are hindered or assisted. The tetanus bacillus, when placed in suitable media, will not grow except in the absence of oxygen; but place it under the same conditions, together with a micro-organism which actively assimilates oxygen, and the two in association will grow in the presence of air. As a rule, when tetanus bacilli are driven into the flesh by a dirty nail or blank cartridge plug, aërobic bacteria are driven in also and so help to further infection by using up the free oxygen, thus introducing an anaërobic environment.

The influenza bacillus is a striking example of the special requirements of certain bacteria. On media it will thrive in pure culture only in the presence of hæmoglobin.

It is evident, therefore, that for each variety of organism there are special conditions requisite for growth, and that a temperature, degree of acidity, kind of food, supply of oxygen, etc., suitable for one may be utterly unsuitable for another; that, still further, when two organisms grow together one may so alter some of these conditions as to render unsuitable ones suitable, and *vice versa*.

Let us now consider some of the facts which have been observed concerning the growth of bacteria in the living body as contrasted with culture media. In the first place, it has been learned, as will be described in the latter part of the book, that each variety of bacteria can incite only certain types of infection. Indeed, because of this fact, the majority of bacteria which excite disease can be traced back for thousands of years by means of the records, these parasitic bacteria breeding true and keeping distinct from the great mass of bacteria occurring in the air, water, and soil.

Parasitic bacteria have gradually adapted themselves not only to certain species of animals, but to certain circumscribed areas of the body. Thus the diphtheria bacilli grow chiefly upon the mucous membranes of the respiratory tract, but cannot develop in the blood or in the subcutaneous tissues. The cholera spirilla develop in the inflamed intestinal mucous membrane, but cannot grow in the respiratory tract, blood, or tissues. The tetanus bacilli develop in wounds of the subcutaneous tissues, but cannot grow on the intestinal mucous membranes or in the blood.

Other bacteria find, indeed, certain regions especially suitable for their growth, but under conditions favorable for them are capable of developing in other locations. Thus, the typhoid bacillus grows most luxuriantly in the Peyer patches and mesenteric glands, but also invades the blood, spleen, and other regions. The tubercle bacillus often remains localized in the apex of a lung or a gland for years, but may at any time invade many tissues of the body. The gonococcus finds the mucous membrane of the genitourinary tract most suitable for its development, but also frequently is capable of growth in the peritoneum and even sometimes in the general circulation. The pneumococcus develops most readily in the lungs, but also invades the connective tissues, serous membranes, and the blood.

All these bacteria, although ordinarily increasing only in the body of man, can be grown on suitable dead material.

There are organisms which, in so far as we know, find the bodies of human beings or animals the only fit soil for their growth. These are strictly the true parasites. The bacillus of leprosy until recently classed with these has just been made to grow on artificial culture media (see Sec. II, under leprosy).

Adaptation of Bacteria to the Soil upon which They are Grown.—Those bacteria which grow both in living and dead substances vary from time to time as to their readiness to develop in either the one or the other. As a general rule, bacteria grown in any one medium become more and more accustomed to that and other media more or less analogous to it, while, on the other hand, they are less easily cultivated on media widely different from that in which they have developed. Thus we had a culture of tubercle bacilli which, after having grown for three years in the bodies of guinea-pigs, would grow only with great difficulty on dead organic matter, while a bacillus which was obtained

from the same stock, but grown since on bouillon, will no longer increase in the animal body. From the same stock, therefore, two varieties have developed, the one having lost and the other gained in ability to develop as a parasite.

Local Effects Produced by Bacteria and Their Products.—After the bacteria gain entrance to a suitable part of the body and find conditions favorable for growth, there is a certain lapse of time before sufficient bacterial poisons have accumulated to cause by the action on the tissue noticeable disturbance. This is called the period of incubation. Its length depends on the amount, kind, and virulence of the microörganisms introduced and the tissue invaded. The incubation period over, we note the course of the local and general lesions excited by the specific and general poisons. The extent to which this will progress depends, on the one hand, on the characteristics of the invading microörganisms; on the other, on the characteristics of the tissues invaded.

The local effects of the bacterial poisons upon the cells give rise to the various kinds of inflammation, such as serous, fibrinous, purulent, croupous, hemorrhagic, necrotic, gangrenous, and, finally, proliferative. Some bacteria incite specific forms of inflammation along with those common to many bacteria; others produce, so far as we can detect, no peculiar form of lesions.

Thus inflammation and serous exudation into the subcutaneous tissues follow injections of the pneumococcus or anthrax bacillus. The development of the streptococcus or pneumococcus in the endocardium or pleural cavity is followed by a serous exudation, frequently with more or less fibrin production. The formation of pus results more especially from the streptococcus, pneumococcus, and staphylococcus; but nearly all forms of bacteria, when they accumulate in one locality, may produce purulent inflammation. The colon, typhoid, and influenza bacilli frequently cause the formation of abscesses.

Catarrhal inflammation, with or without pus, follows the absorption of the pruducts of many bacteria, such as the gonococcus, pneumococcus, streptococcus, and influenza bacillus, etc. The hemorrhagic exudation seen in pneumonia is usually due to the pneumococcus; it is observed also in other infections. Cell necrosis is produced frequently by the products of the diphtheria and of the typhoid bacilli and by those of other bacteria. Specific proliferative inflammation follows the localization of the products derived from the tubercle bacillus and the leprosy bacillus.

Not only can the poisons of one species of bacteria, according to the tissues attacked, produce several forms of inflammation, but the same organism will vary as to its mode and extent of invasion; this depending, first, upon its own characteristics, at the time, as to virulence, etc., and, second, upon the conditions in the infected animal, such as its health and power of resistance, the location of infection, and the circumstances under which the animal is kept. Such varia-

tions, therefore, are in no case specific, for different poisons will produce changes which appear identical.

Manner in which Bacteria Produce Injury.—Bacteria produce serious mechanical injury only when they exist in such enormous numbers or bunched together as to interfere mechanically with the circulation or cause minute thrombi, and later emboli, which finally produce infarction and abscesses in different parts of the body. Even these dangerous effects are almost wholly due to the chemical substances given off, which are more or less directly poisonous. Some portion of the protoplasm of almost every variety of bacteria acts as an irritant to tissues and combines with some of the substance of some of the body cells, and the protoplasm of most exerts a positive chemotaxis.

These poisonous products, as already described in the previous chapter, can often be separated from the culture fluid in which the bacteria have grown, or they can be extracted from the bacteria. Injected into animals these products cause essentially the same cellular lesions as are produced by the bacteria when they develop in the animal body. The substances contained in or produced by the bacteria, with few exceptions, attract the leukocytes, and when great masses of bacteria die suppuration usually follows.

General Symptoms Caused by Bacterial Poisons Absorbed into the Circulation.—Fever is produced, under favorable conditions, by all bacterial poisons. A prime requisite is that sufficient poisons be absorbed; on the other hand, they must not be absorbed with such rapidity as to overwhelm the infected host, for a moderate dose may raise the temperature, while a very large dose lowers it, as occurs sometimes when a very large surface, such as the peritoneum, is suddenly involved. The fever itself has no known antibacterial effect, but this effect may be some part of the reaction of the tissues which in other portions gives rise to the antitoxins and bactericidal substances. It is also a sign that the body cells as a whole are not yet overwhelmed by the infection.

With few exceptions the bacterial poisons produce an increase in the number of leukocytes and a lessening in the amount of hæmoglobin in the blood. In uncomplicated infection with typhoid bacilli there is a hypoleukocytosis. The different varieties of leukocytes are increased in varying proportions in different infections. The red-blood cells are directly injured by a number of bacterial substances. The deleterious effects on the nutrition are partly due to the direct effect of the poison and partly to the diseased conditions of the organs of the body, such as the spleen, kidney, and liver. Degeneration of the nerve cells is frequently noticed after infectious diseases; especially is this true of diphtheria. Several bacterial poisons have been found to produce convulsions; the best example of this is the tetanus toxin.

Influence of Quantity in Infection.—With pathogenic bacteria the number introduced has an immense influence upon the probability of infection taking place.

If we introduce into a culture medium containing some fresh human blood or serum a few bacteria it is probable that they will all die because of the presence of sufficient bactericidal substance in the blood to destroy them; whereas if a greater number are introduced, while there will at first be a great diminution of these, those that die, having combined with the bactericidal substances in the serum, neutralize them; then those bacteria which survive begin to increase, and soon they multiply enormously. The same is true for parasitic bacteria in the body. A few only gaining entrance, they may die; a larger number being introduced, some may or may not survive; but if a still greater quantity is injected it is almost certain unless the animal is immune that there will be some surviving members, which will begin to proliferate and excite disease.

Variation in Degree of Virulence Possessed by Bacteria.—Bacteria differ, as has already been stated, as to the ease and rapidity with which they grow in any nutritive substance and the amount of poison they produce. Both of these properties not only vary greatly in different members of the same species, but each variety of bacteria may to a large extent be increased or diminished in virulence. The septicæmic class of bacteria when grown in the body fluids seem to gradually develop the power to elaborate protective substances in their own bodies or produce cells with less substance having affinity for the bactericidal bodies of the blood, and thus become less vulnerable.

With those bacteria whose virulence is great a very few organisms will produce disease almost as quickly as a million, allowance only being made for the short time required for the few to become equal in number to the million. At the other extreme of virulence, however, many millions may have to be introduced to permit of the development of any of the organisms in the body. With these bacteria we are thus able to produce either no effect whatever, or a local effect, or in some cases a general septicæmia, by regulating the amount of infection introduced.

Somewhat distinct, again, from that class of bacteria which multiply rapidly are those which, like the tubercle and leprosy bacilli, which while surely developing infection, increase more slowly. Here increase of virulence is shown, as before, by the production of disease through the introduction of very small numbers into the body, but increase in rapidity of development cannot progress except to within certain limits. A single streptococcus may, through its rapid multiplication, produce death in eighteen hours; a single tubercle bacillus, on the other hand, cannot produce sufficient numbers in less than two weeks. The virulence of the septicæmic class of bacteria is not at all the same when measured in different animals, and it is largely for this reason at the virulence in test animals does not usually correspond with the verity of the case from which the organism was derived.

Experimental Increase and Decrease in Toxicity and Virulence.—he power to produce toxin can be taken from bacteria by growing hem under adverse circumstances, such as cultivation at the maximum

temperature at which they are capable of development. Some bacteria are easily attenuated; others are robbed of their virulence only with great difficulty. Increase of toxin production is more difficult, and it is only possible to obtain it to a certain extent. The means usually employed are the frequent replanting of cultures. But with all our efforts we are usually only able to restore approximately the degree of toxin formation which the cultures originally possessed. The adaptation of bacteria to any nutritive substance, living or dead, so that they will grow more readily, is more easily brought about, provided they will grow at all. The streptococcus from erysipelas and the pneumococcus from pneumonia are typical of this class of bacteria. Inoculate a rabbit with a few streptococci obtained from a case of human sepsis, and, as a rule, no result follows; inject a few million, and usually a local induration or abscess appears; but if one hundred million are administered septicæmia develops. From this rabbit now inoculate another, and we find that a dose slightly smaller suffices to produce the same effect; in the next animal inoculated from this, still less is required, and so on, until in time, with some cultures, a very minute number will surely develop and produce death. With other cultures this increase does not take place. The same increase in virulence can be noted when septic infection is carried in surgery or obstetrics from one human case to another. By allowing bacteria to continue to develop under certain fixed conditions they become accustomed to these conditions, and less adapted to all that differ.

Mixed Infection.—The combined effects upon the tissues of the products of two or more varieties of pathogenic bacteria, and also of the influence of these different forms on each other, are of great importance in the production of disease. The infection from several different organisms may occur at the same time, or one may follow the other or others—so-called secondary infection. Thus, an abscess is often due to several forms of pyogenic cocci. If a fresh wound is infected from such a source the inflammation produced will probably be caused by all the varieties present in the original infection. Peritonitis following intestinal injuries must necessarily be due to more than one variety of organism. Thus, whenever two or more varieties of bacteria are transferred to a new soil, mixed infection takes place if more than one is capable of developing in that locality.

Forms of infection which are allied to both mixed and secondary infection are those occurring in the mucous membranes of the respiratory and digestive tract. In these situations pathogenic bacteria of slight virulence are always present even in health. Thus, in the upper air passages there are usually found streptococci, staphylococci, and pneumococci. When through a cold, or the invasion of another infective agent, as the diphtheria bacillus or the virus of smallpox or scarlet fever, the epithelium of the mucous membrane of the throat is injured or destroyed, the pyogenic cocci already present are now enabled in this diseased membrane to grow, produce their poison, and even invade deeper tissues. The intestinal mucous membrane is

invaded in a similar way by the colon bacilli and other organisms after injury by the typhoid or dysentery bacilli or cholera spirilla. Generally speaking, all inflammations of the mucous membranes and skin contain some of the elements of mixed infection. Blood infection, on the other hand, is usually due to one form of bacteria, as even when several varieties are introduced, only one, as a rule, is capable of development. The same is true to a somewhat less extent of inflammation of the connective tissue. The additional poison given off by the associated bacteria aid infection by the primary invaders by causing a lowering of the vital resistance of the body. In some cases the secondary infection is a greater danger than the primary one, as pneumococcic bronchopneumonia in laryngeal diphtheria or streptococcic septicæmia in scarlet fever and smallpox.

The bacteria are also at times directly influenced by the products of associated organisms. These may affect them injuriously, as, for example, the pyogenic cocci in anthrax; or they may be necessary to their development, as in the case of anaërobic bacteria. Not infrequently the tetanus bacilli or spores would not be able to develop in wounds were it not for the presence of aërobic bacteria introduced with them. This is shown outside the body, where tetanus bacilli will not grow in the presence of oxygen unless aërobic bacteria are associated with them. Again, it is found that the association of one variety with another may increase its virulence. Streptococci are stated to increase the virulence of diphtheria bacilli, but here it is probably the loss of resistance of the tissues because of the streptococcic poison. On the other hand, the absorption of the products of certain bacteria immunizes the body against the invasion of other bacteria, as shown by Pasteur that attenuated chicken-cholera cultures produce immunity against anthrax. In intestinal putrefaction harmless varieties of bacteria may be made to crowd out dangerous ones.

Tissue Characteristics Influencing the Entrance and Growth of Bacteria.—The Skin.—The skin is a poor soil for bacteria and is a great protection against the penetration of microörganisms. When they do penetrate, it is through the diseased glands, or more often through some unobserved wound. The bacterial toxins are, when at all, absorbed to a slight extent through the skin.

There is an apparent exception to the above statements in the fact that the pyogenic staphylococci and sometimes the streptococci exist upon the skin or in it between its superficial horny cells, some exceptional circumstances, such as wounds or burns, being required to allow the organisms to penetrate deeper. The cutaneous sweat glands, and the hair follicles with their appended sebaceous glands, may allow entrance of infection, as various incidents may lead to the introduction and retention of virulent microörganisms. When this occurs the retained products may lead to necrosis of the epithelium and thus allow the bacteria to penetrate to the deeper tissues. The secretion of the sebaceous glands appears to be little, if at all, bactericidal, but the perspiration, on account of the acidity, is slightly so.

Subcutaneous Connective Tissues.—Many bacteria cannot develop in the connective tissues and others produce a milder infection there than elsewhere. Others develop readily.

The Mucous Membranes.—The moist condition of the surface of the membranes and their frequent contact with irritating substances render them liable to bacterial infection. Bacteria, such as the pneumococci and streptococci, reproducing themselves in it become somewhat attenuated. The mucous membranes are protected by the cleansing produced by the flow of the secretion and by its slight germicidal action. In infancy the membranes are readily infected by gonococci and later by pneumococci, by the Koch-Weeks bacillus and others. The mucous membranes of the nasal cavity are somewhat cleansed by the nasal secretion. The deeper portions of the nasal cavity are usually the seat of streptococci and other bacteria, while the extreme anterior portion contains saprophytic bacteria from the air. The mouth in a person in health is cleansed by the feebly bactericidal saliva. When the teeth are decayed many varieties of bacteria abound. Many of these are difficult to cultivate. The bacteria, such as the diphtheria bacilli, streptococci, etc., rarely invade the mucous membrane of the tongue or mouth.

The tonsils with their crypts are usually the seat of the pyogenic cocci and are readily infected by the diphtheria bacilli and others. Whether the absolutely intact epithelium allows the passage of these bacteria is disputed, but the probability is that it does. With the slight pathological lesions usually present it undoubtedly does.

The Lungs.—Most inhaled bacteria which pass the larynx are caught in the bronchi. Many of these are gradually removed by the ciliated epithelium. Both the alveolar epithelial cells and the leukocytes which enter the air sacs and bronchioles have been shown to take up bacteria. The normal lung is, therefore, rapidly freed of saprophytic and many parasitic bacteria. When subjected to deleterious influences, such as exposure to cold, the lung tissues seem to lose their protective defences and become subject to infection.

The Stomach.—The pure gastric juice, through the hydrochloric acid it contains, is able to kill most non-spore-bearing organisms in a short time, but because of neutralization through food, or because the bacteria are protected in the food, many of them pass into the intestines. Tubercle, typhoid, colon, and dysentery bacilli, when fed by the mouth with food, readily pass beyond the stomach. Certain acidophilic germs, as well as yeasts and torulæ, seem to grow in the gastric secretion; these are largely non-pathogenic. Perforation of the stomach is usually followed by peritonitis, because of the irritant effect of the gastric juice and the presence of bacteria which are temporarily retained. The gastric juice alters tetanus and diphtheria toxins. The toxicity of some poisons, such as occur in decayed meat, are not destroyed. The stomach is exceptionally free from bacterial inflammations.

Intestines.—The bile is feebly bactericidal for some bacteria, but,

on the whole, the intestinal secretions have little or no germicidal power. The number of bacteria increases steadily from the duodenum to the head of the colon, and diminishes slightly from the upper to the lower end of the colon. The pancreatic juice destroys many of the toxic bacterial products. The presence of the bacilli of the colon group, of streptococci, etc., does not often lead to any inflammatory condition in the normal intestines of healthy persons. In children suffering from the prostrating effects of heat they are apt to excite inflammatory changes. Even pathogenic bacteria, such as the typhoid, dysentery, and tubercle bacilli, may pass through the whole length of the healthy intestines without inciting inflammations. Slight lesions aid the passage of bacteria to the deeper structures. Tubercle bacilli and other pathogenic bacteria may pass through the intestinal wall to the lymph and cause distant infection without leaving any trace of their passage.

Importance of Location of Point of Entry of Bacteria.—Most bacteria cause infection only when they gain access to special tissues and must, therefore, enter through certain portals. This fact is of immense importance in the transmission or prevention of disease. Thus, for example, let us rub very virulent streptococci, typhoid bacilli, and diphtheria bacilli into an abrasion on the hand. The typhoid bacillus produces no lesion, the diphtheria bacillus but a very minute infected area, but the streptococcus may give rise to a severe cellulitis or fatal septicæmia. Now place the same bacteria on an abrasion in the throat. The typhoid bacillus is again harmless; the diphtheria bacillus produces inflammation, a pseudomembrane, and toxæmia, and the streptococcus causes an exudate, an abscess, or a septicæmia. Finally, introduce the same bacteria into the intestines, and now it is the typhoid bacillus which produces its characteristic lesions, while the streptococcus and diphtheria bacillus are usually innocuous.

It we tried in this way all the parasitic bacteria we would find that certain varieties are capable of developing, and thereby exciting disease, only on the mucous membrane of the throat, others of the intestine, others of the urethra; some develop only in the connective tissues or in the blood, while others, again, under favorable conditions, seem able to grow in or upon most regions of the body.

The Dissemination of Disease.—The spread of infection is influenced by: 1. The number of species of animals subject to infection.

Many human infectious diseases do not occur in animals, and many animal infections are not found in man. Thus, so far as we know, gonorrhœa, syphilis, measles, smallpox, typhoid fever, etc., do not occur in animals under ordinary conditions; while tuberculosis, anthrax, glanders, hydrophobia, and some other diseases are common to both man and animals.

2. The quantity of the infectious material and the manner in which it is thrown off from the body.

In diphtheria, typhoid fever, cholera, pulmonary tuberculosis, septic endometritis, influenza, and gonorrhœa, enormous numbers of infec-

tious bacteria are cast off through the discharges from the mouth, intestines, and genitourinary secretions, causing great danger of infection. On the other hand, in tuberculous peritonitis, streptococcic meningitis, and endocarditis, gonorrhœal rheumatism, and the like there is little or no danger of infecting others, as few or no bacteria are cast off.

3. The resistance of the infectious bacteria to the deleterious effects of drying, light, heat, etc.

In this case the presence or absence of spores is of the greatest importance. The spore-bearing bacilli such as tetanus, anthrax, etc., being able to withstand destruction for a long time, retain their power of producing infection for months or even years after elimination from the body. The bacteria which form no spores show great variation in their resistance to outside influences. Some of these, such as the influenza bacilli and the gonococci, the virus of syphilis and hydrophobia, are extremely sensitive; the pneumococci, cholera spirilla, glanders bacilli, etc., are a little hardier; then follow the diphtheria bacilli, and after them the typhoid and tubercle bacilli and the staphylococci.

4. The ability or the lack of ability to grow outside of the infected tissues.

Such bacteria as the pneumococcus, tubercle, influenza, diphtheria, glanders, and leprosy bacilli do not develop, as far as we know, outside of the body under ordinary conditions. Under exceptional circumstances, as in milk, some may develop. Others, again, such as the streptococcus and staphylococcus, typhoid and anthrax bacillus, the cholera spirillum, and some anaërobes, may develop under peculiar conditions existing in water or soil.

While for the pathogenic bacteria, as a rule, the saprophytes met with in the soil and water are antagonistic, yet in some cases—and especially is this true of the anaërobic bacteria—they are helpful. Such bacilli as tetanus are believed to require the association of aërobic bacteria to permit of their development in the soil in the presence of oxygen.

5. **Bacteria Carriers.**—Human bacteria develop in these cases in or upon some portion of the skin or mucous membrane, either after or before disease, and without causing infection. As complete a knowledge as possible of this saprophytic development in man of parasitic bacteria is necessary if we are to combat the spread of infection. In the superficial layers of the epithelium and on the surface of the skin we find the different pyogenic cocci, which are capable of infecting a wounded or injured part or causing inflammation in the glands. Acne, the pustules in smallpox, the pus on a burned surface, boils, etc., all come from these pyogenic cocci. In surgical cases the skin has to be as thoroughly disinfected as possible, to prevent the formation of stitch-hole abscesses and wound suppuration.

In the secretion of the mucous membrane covering the pharynx and nasopharynx there is always an abundance of bacteria. In throats

examined in New York City, streptococci, staphylococci, and pneumococci are found in almost every instance, and even in the country they are often present. In the anterior nares there are fewer parasitic bacteria than in the posterior portions. The nasal secretion is only very slightly, if at all, bactericidal. Many other varieties of bacteria, such as the meningococci and the influenza bacilli, are probably often present in small numbers. In those constantly in contact with cases of diphtheria, and in those convalescent from diphtheria, virulent diphtheria bacilli are frequently found in the throat.

After convalescence from typhoid fever, from one to three per cent. remain bacillus carriers for months or years, the bacilli continuing to develop in the bile passages and are passed with the feces.

Lower Animals.—The lower animals, as a rule, do not retain in their bodies bacteria pathogenic for human beings, but, as direct carriers of infection, they are important factors. Biting insects, especially, such as fleas, ticks, bedbugs, lice, flies, and mosquitoes are a source of danger (see under Protozoa for these insects acting as intermediate hosts for certain Protozoa). Flies and other insects may convey organisms which are simply attached to their feet or other surfaces of their bodies.

Bacterial Autoinfection.—When the intestinal canal is injured, or its circulation hindered by strangulation, etc., *Bacillus coli* and some other bacteria may penetrate through the injured walls and cause peritonitis or general infection. Under certain conditions, as during the debility due to hot weather, the bacteria in the intestines cause, through their products, irritation, and in children even serious intestinal inflammation. Long after an acute gonorrhœa has passed gonococci may remain in sufficient numbers to cause a new inflammation or produce infection in others. A cystitis may run on chronically for years, and then suddenly become acute or spread infection to the kidneys. A persistent gonorrhœal vaginal infection may lead to a gonorrhœal endometritis, or peritonitis or salpingitis, under suitable conditions. The staphylococci in the skin and the colon bacilli and pyogenic cocci in the fecal discharges may also be carried into the bladder and uterus and produce septic infection. Persons carrying diphtheria bacilli in their throats or typhoid bacilli in their gall-bladder may, under predisposing conditions, develop diphtheria or typhoid fever.

In nearly all cases of infection the products of bacterial growth are absorbed into the blood, and along with them a few bacteria also, even when they do not reproduce themselves in it. The greater the extent of the infection and the more deep-seated it is, the greater is the amount of absorption. The bacteria enter the blood, according to Kruse, by (1) passive entrance through the stromata of the capillary walls; (2) carriage into the blood in the bodies of leukocytes; (3) growth of the bacteria through the walls of the vessels; (4) transmission of the bacteria through the lymph glands placed between the lymph- and blood-vessels.

When bacteria are abundant in the blood they become fixed in the capillaries of one or all of the organs, especially of the liver, kidneys, spleen, and lungs, and then directly or by means of the leukocytes, which penetrate the capillary walls, they pass into the tissues and substance of the organs. They thus reach the lymph channels and glands, or gain entrance into the gall-bladder, saliva, etc., or press through the epithelium, as in the alveoli of the lungs; more rarely they pass through the kidney tissue into the urine, as in typhoid fever, though some deny that this can happen unless there is a previous inflammation of the kidneys.

Elimination of Bacteria through the Milk.—The passage of bacteria through the breast is important, from the fact that milk is so largely used as food. Observers have reported the finding of tubercle bacilli in cow's milk when the gland itself was intact and the animal tuberculous. Some authorities have put its presence in milk, under these circumstances, as high as 50 per cent. of the cases. This, in our experience, is undoubtedly too high. The fact that tubercle bacilli swallowed with the sputum are passed alive in the fæces explains the frequent occurrence of bacilli in the milk of cows without udder tuberculosis because of the contamination of the milk with manure. They are undoubtedly present, however, in the milk of some animals in which tuberculous disease of the gland could not be demonstrated. In these cases lymph glands adjacent to the udder are usually infected. The finding of streptococci and staphylococci is due probably in the majority of cases to the infections taking place as the milk is voided, for the epithelium at the outlet of the lacteal ducts is always infected with staphylococci, and frequently with streptococci, which have often been received from the mouth of the sucking infant.

Elimination of Bacteria by the Skin and Mucous Membranes.—Whether bacteria pass from the blood by the sweat is a mooted point. The skin is always the seat of the staphylococcus and frequently of other bacteria, so that it is difficult to determine in any given case the origin of the bacteria found in the sweat. Many observers have reported the passage of bacteria from the blood through the mucous membrane. These, as shown by Hess, are few in number, however. Bacteria are sometimes eliminated through the urine, but here, as a rule, when great numbers of organisms are found, it is due to development in the bladder. The removal of the poisonous products of bacteria by the kidneys, intestines, etc., on the contrary, is of great advantage to the organism.

CHAPTER XI.

THE ANTAGONISM EXISTING BETWEEN THE FLUIDS AND CELLS OF THE LIVING BODY AND MICRO-ORGANISMS.

THAT certain races of animals and men, and certain individuals among these, are more refractory to disease than others is a fact which has long been known. Experience and observation have taught us, further, that the same individuals are at one time more resistant to disease than at another. This inborn or spontaneous refractory condition to an infectious disease is termed natural immunity, in contradistinction to that acquired by recovery from infection.

In regard to variations in susceptibility, certain known facts have been accumulated. Thus, cold-blooded animals are generally insusceptible to infection from those bacteria which produce disease in warm-blooded animals, and *vice versa*. This is partly explained by the inability of the bacteria which grow at the temperature of warm-blooded animals to thrive at the temperature commonly existing in cold-blooded animals. But differences are observed not only between warm-blooded and cold-blooded animals, but also between the several races of warm-blooded animals. The anthrax bacillus is very infectious for the mouse and guinea-pig, while the rat is not susceptible to it unless its body resistance is reduced by disease and the amount of infection is great. The inability of the microörganism to grow in the body of an animal does not usually indicate, however, an insusceptibility to its poison; thus, for instance, rabbits are less susceptible than dogs to the effects of the poison elaborated by the pneumococci, but these bacteria develop much better in the former than in the latter. In animals, as a whole, it is noticed experimentally that the young ones are less resistant to infection than the older and larger ones.

The difficulty experienced by the large majority of bacteria in developing in the tissues of the healthy body can be to a great extent removed by any cause which lowers the general or local vitality of the tissues. Among the causes which bring about such lessened resistance of the body are hunger and starvation, bad ventilation and heating, exhaustion from over-exertion, exposure to cold, the deleterious effects of poisons, bacterial or other, acute and chronic diseases, vicious habits, drunkenness, etc. Purely local injuries, such as wounds, contusions, etc., give a point of entrance for infection, and tissue of less resistance, where the bacteria may develop and through their poison produce adjacent injury and so predispose to further bacterial invasion in much the same way as the heat of the forest fire

dries the green trees in front of it and so prepares them to ignite. Local affections, such as endocarditis, may also afford an area of lessened resistance. The presence of foreign bodies in the tissues in like manner predisposes them to bacterial invasion. Interference with free circulation of blood and retention in the body of poisonous substances which should be eliminated also tend to lessen the vitality. In these and other similar ways animals which are otherwise refractory may acquire a susceptibility to disease.

Increase of Resistance by Non-specific Means.—All conditions which are favorable to the health of the body increase its resistance, and thus aid in preventing and overcoming infection. The internal use of antiseptics against bacteria is so far unsuccessful, for the reason that an amount still too small to inhibit bacterial growth is found to be poisonous to the tissue cells. The efficacy of quinine in malaria and mercury in syphilis are, possibly, exceptions to the rule, but in both cases we are dealing with animal parasites, not with bacteria. Such substances as leukocytic extract, nuclein, and similar organic substances contained in blood serum, when introduced into the body in considerable quantity, aid somewhat in inhibiting or preventing the growth of many bacteria. Even bouillon, salt solution, and small amounts of urine have a slight inhibitory action. The hastening of elimination of the bacterial poisons by free intestinal evacuation and encouragement of the functions of the skin and kidneys are also of some avail. The enzymes formed by certain bacteria have been found to exert a slight bactericidal action not only on the germs which have directly or indirectly produced them in the body, but also on other varieties. None of these enzymes are sufficiently protective to be of practical value, nor are they equal in power to the protective substances formed by the tissues from the bacterial products.

Use of Local Treatment in Limiting Bacterial Invasion.—The total extirpation of the infected area by surgical means, if thoroughly carried out, removes the bacteria entirely; but, unfortunately, this procedure is rarely possible. When incomplete it is frequently helpful; but it may be harmful, for by creating tissue injury and exposing fresh wounded surfaces to infection it may lead to the further development of the disease. In some cases, however, like anthrax and infection from bites of rabid animals, almost complete removal of the virus, either by the knife or thorough cauterization, will prevent a general infection or so lessen the number of bacteria in the body as to allow the bactericidal element of its fluids to exterminate them. So also in tetanus, the invasion being limited, surgical interference may be of great use by removing not only the bacilli themselves, but also that portion of their poison which has not as yet been absorbed from the tissues. The beneficial effects of opening an abscess, or cleansing and draining the pleural, peritoneal or uterine cavities are well known. The retention of the poisonous products of the bacteria leads to their absorption, and then through their combining with some of the tissue cells and with the protective substances of the adjacent fluids

the tone of the tissues is lowered at the same time that bactericidal substances have been neutralized. This enables the gems to penetrate into tissues which would otherwise resist them. The mechanical effect of pressure on the walls of an abscess by its contents also aids absorption of toxins and bacterial progress. Local bleeding and the application of cold probably act by lessening absorption. The application of warmth increases the blood flow to the part, and so, when the general blood supply is bactericidal, as it often is, it acts favorably on the inflammation. A similar effect of operative interference is noticed in the frequently observed beneficial result of laparotomy in tuberculous peritonitis.

Antiseptic solutions have the power of cleansing and rendering sterile the surfaces of a wound—that is, of lessening the introduction of infection. After infection has taken place, however, it is doubtful whether antiseptic washing has much more direct influence than simple cleansing, and it certainly can have no bactericidal effect at any distance from the surface, either direct or indirect. Certain infectious diseases which are comparatively superficial are probably benefited by antiseptic solutions; such are gonorrhœa, diphtheria, and other inflammation of the mucous membranes. Even here, however, it is impossible to do more than disinfect superficially, and in some cases any irritation of the tissues is apt to do more harm than good. In the superficial lesions of syphilis and tuberculosis the local use of antiseptics is sometimes of great value. In these diseases the irritant effects of the antiseptics which stimulate the tissues may also be beneficial.

Specific Immunity, or a Condition of the Body which Prevents the Development in it of One Variety of Microörganisms or Renders it Unaffected by Their Bacterial Poisons.—The invasion of the body by almost every variety of microörganism is followed, if death does not quickly ensue, by conditions which for a variable period and to a variable degree are deleterious to the further growth of that variety. This more or less pronounced specific immunity may be created in various ways:

1. Through recovery from disease naturally contracted or from infection artificially produced. According to the nature of the invading microörganism this immunity may be slight, as after recovery from erysipelas or pneumonia, marked for a limited period of time, as in diphtheria and typhoid fever, or prolonged, as after scarlet fever or syphilis.

2. By inoculation with microörganisms attenuated by heat, chemicals, or other means. In this case an infection of the animal is produced, of moderate severity, as a rule, and the immunity is not quite as marked and lasting as after recovery from a more serious attack; but it is, nevertheless, considerable. The inoculation of sheep with the attenuated anthrax bacillus and the use of vaccination with cow-pox in man are examples of this method.

3. By the injection of the living organisms into tissues where develop-

ment will not take place, as the injection of diphtheria bacilli, typhoid bacilli or cholera spirilla into the subcutaneous tissues. Here the destruction of the bacteria with the absorption of their products causes a mild chemical poisoning, with considerable resulting immunity.

4. By the injection of the dead bodies of bacteria or of the chemical products which they elaborate and discharge into the surrounding culture media during their life. This produces a less marked immunity than when the living culture is used, but the method is a safer one.

5. By the injection of the blood serum of animals which have previously passed through a specific disease or have been inoculated with the bacterial products. The first, probably, to think of the possibility of effecting this was Raynaud, who in 1877 showed that the injection of large quantities of serum derived from a vaccinated calf into an animal prevented its successful vaccination. The results obtained by Behring and Kitasato upon diphtheria and tetanus, where the serum neutralized the poisons rather than the direct development of the bacteria, gave a still greater impetus to these investigations.

Suitable animals after repeated infections gradually accumulate in their blood considerable amounts of these protective substances, so that very small amounts of serum inserted in another animal will inhibit the growth of the bacteria or neutralize their products. Thus, 0.1 c.c. of a serum from a horse frequently infected by the pneumococcus will prevent the development in the body of a rabbit of many thousand times the fatal dose of very virulent pneumococci, and a few times a fatal dose of less virulent ones, the actual number as well as the virulence of the bacteria affecting the protective value of the serum.

These protective substances are found also in other fluids of the body than in the blood; they occur, indeed, in the substance of many cells to a greater or less extent.

The immunity produced by these five methods affects the entire body, as is natural, since the blood into which they are absorbed is distributed everywhere. The protective substances pass from the blood through the walls of the capillaries and finally find their way to the lymph and back to the blood. When the immunity is but slight, infection may take place in the more sensitive regions or where a large number of bacteria have gained access, and still be impossible in those tissues having more natural resistance or slighter infection.

Passive as Contrasted with Active Immunity.—After the immune serum is injected into man the immunity is greatest at the time of its reception into the blood. This, of course, is instantaneous after an intravenous injection, but only after eight to sixteen hours when given subcutaneously, and then declines, being rather quickly (in several months or weeks, according as to whether or not the serum is injected into the same species of animal as the one from which it was drawn) almost entirely lost, so that repeated injections are required to maintain the immunity. This passive immunity is distinctly in contrast to the active immunity acquired after the introduction of bacteria or bacteria products, where the tissues of the organism, in ways to us

unknown, throw out, in response to the bacterial stimulus, inhibitory or antitoxic substances. Here immunity is actually lessened for one or two days, and then is increased, and reaches its height a week or ten days after the injection, and then continues for a week or two, when it slowly declines again and is lost after several months or years.

Testing of Protective Power of Antibacterial and Antitoxic Sera.—The serum is tested by mixing it with a certain number of times the fatal dose of a culture or its toxins whose virulence or toxicity is known, and then injecting this under the skin, in the vein, or into the peritoneum, according to the nature of the substance to be tested. The main point is that some definite method be carried out by which the relative value of the serum can be judged in comparison with other serums. As a rule, the value is stated in the number of fatal doses of culture or toxin which a fraction of a cubic centimetre of serum will prevent from destroying the animal. It is well to remember that with a living germ a multiple of a fatal dose is not as much more severe than a single dose as the figure would suggest. One thousand times a fatal dose of a very virulent microörganism will be neutralized by several times the amount of serum which a single fatal dose requires, since in the case of very virulent bacteria, whose virulence is due to their ability to increase, it is not the organisms which are introduced that kill, but the millions that develop from them.

Limitation of Curative Power of Serums which act Directly Against Microörganisms.—As a rule, the serum has to be given before the bacteria introduced into the body have multiplied greatly. After that period has elapsed the serum usually fails to act. This is partly because the bactericidal and antitoxic substances of the serum are insufficient in amount and partly because suitable antibodies develop for only a portion of the varied types of poison produced by bacterial cells.

Practical Therapeutic Value of Bactericidal Sera.—The use of serums having specific protective properties has been tried practically on a large scale in man as a preventive of infection. In susceptible animals injections of some of the very virulent bacteria, as pneumococci, streptococci, meningococci, and typhoid bacilli, can be robbed of all danger if small doses of their respective serums are given before the bacteria have increased to any great extent in the body. If given later they are usually ineffective. For some bacteria, such as tubercle bacilli, no serum has been obtained of sufficient power surely to prevent infection. Through bactericidal serums, therefore, we can immunize against many infections, and even stop some just commencing; but as yet we cannot cure an infection which is already fully developed, though even here there is reason to believe that we may possibly prevent an invasion of the general system from a diseased organ, as by the pneumococcus from an infected lung in pneumonia. On the whole, the serums which simply inhibit the growth of bacteria without neutralizing the toxins have not given, as observed in practice, conclusive evidence of great value in already developed disease.

Relative Development of Antitoxins and Bactericidal Substances in the Different Infections.—Although the serum of animals which have been infected with any one of many varieties of bacteria is usually both antitoxic and bactericidal, still one form of these protective substances is usually present almost alone; thus antitoxic substances are present almost exclusively in animals injected with two species of bacteria which produce powerful specific poisons—viz., the bacilli of diphtheria and tetanus. When the toxins of either of these are injected in small amounts the animals after complete recovery are able to bear a larger dose without deleterious effects. To Behring and Kitasato we owe the discovery that this protecting substance accumulates to such an extent in the blood that very small amounts of serum are sufficient to protect other animals from the effects of the true extracellular toxins.

Except the diphtheria and tetanus bacilli, a few only of the important parasitic bacteria attacking man produce these extracellular toxins in any considerable degree and thus become capable of causing the production in the body of antitoxins, and even these do it to a far less extent than those of tetanus and diphtheria. Following them are the dysentery and plague bacilli, and then the cholera spirilla, the typhoid bacilli, the gonococci, meningococci, streptococci, etc. These latter bacteria when injected excite more of the substances which inhibit bacterial growth than of those which neutralize their toxins. The bacillus of symptomatic anthrax and of botulismus and the vegetable poisons ricin, crotin, and abrin also produce specific antitoxins.

Antitoxin a Preventive.—Antitoxin prevents the poisonous action of toxin. It does not restore the cells after they have been injured by the toxin: it is, therefore, like the bactericidal substances, a preventive rather than a cure. We find, experimentally, that a very much smaller amount of antitoxin will neutralize a fatal dose of toxin in an animal, if given before or at the same time, than if given only shortly after it. An animal already fatally poisoned by the toxin is unaffected by any amount of antitoxin.

Stability of Antitoxins.—Kept cool, and protected from access of light and air, the more resistant antitoxins may be preserved sometimes for a year or two with very little deterioration in strength. At other times, however, from unknown causes, they are gradually destroyed, so that there may be a loss of about 2 per cent. per month. Preservatives, such as chloroform, carbolic acid, tricresol, etc., alter antitoxins only very slightly when in dilute solution, but in strong solution they partially destroy them. Heat up to 62° C. does not injure them greatly, but higher temperatures alter them.

Method of Administration.—Antitoxins and bactericidal substances are absorbed by the gastrointestinal tract to a very slight extent only—certainly less than 2 per cent. They must, therefore, be introduced subcutaneously or intravenously to enter the body in appreciable amounts.

CHAPTER XII.

NATURE OF THE PROTECTIVE DEFENCES OF THE BODY AND THEIR MANNER OF ACTION—EHRLICH'S "SIDE CHAIN" AND OTHER THEORIES.

THE fluids and tissues of the animal body under the normal conditions of life are, as we have seen, not only unsuitable for the growth of the great majority of the varieties of bacteria, but even bactericidal to the living organisms.

In seeking to account for the bactericidal property of the blood, which to a greater or less extent affects all bacteria, we cannot find it either in the insufficient or excessive concentration of the nutritive substances, or in the temperature, or in the reaction. We are thus driven to the conclusion that the body fluids and cells contain substances which are deleterious to bacteria.

Bactericidal Properties of the Blood.—The bactericidal effect upon most bacteria of the blood serum, noted by Nuttall in 1888, is now undisputed, and is readily shown by the fact that moderate numbers of bacteria when inoculated into freshly drawn blood usually die soon, and this destruction may be so rapid that in a few hours none of millions remains alive. Even when some of the bacteria survive there is for a time a decrease in the number living. Buchner in 1889 showed that serum heated to 55° lost its destructive power. He believed that in serum there was but a single bactericidal substance and called it alexin.

Pfeiffer in 1894 showed that when an excessive number of cholera spirilla were injected into the peritoneal cavity of a guinea-pig, which had not been immunized to cholera spirilla, they increased and caused death, while in an immunized animal they rapidly disintegrated. He discovered further that if a little of the serum of an immunized animal is injected into the peritoneum of an untreated one, destruction of bacteria takes place. He thus showed that there was a great increase in the bactericidal power of a serum after immunization for the species of bacteria used in immunization. Metchnikoff then showed that the immunized serum added to peritoneal fluid in the test-tube would have the same effect on the spirilla.

Bordet in 1895 reported that defibrinated blood filtered free of blood cells could be used to replace the peritoneal fluid and that if to a serum from an immunized animal, which had lost through age its bactericidal power, fresh serum from an untreated animal was added, the serum regained its destructive powers, *i. e.*, it was activated, although the fresh serum by itself had almost no effect. These observations of Pfeiffer and Bordet indicated clearly that two types of substances were required

to destroy cells. Both of these were present in fresh immune serum, one of which was stable and more or less specific, and the other unstable and non-specific. The latter was proven to be present in all blood, while the former existed, except in minute amount, only in the blood of the immunized. The number of bacteria introduced in a germicidal test is of great importance, for the serum with its contained substances is capable of destroying only a certain number, and after that it has lost its bactericidal properties.

Thus the following test illustrates this:

No. of bacteria in 1 c.c. fluid	Amount of Serum added	Approximate number alive after being kept at 37° C.		
		One hour	Two hours	Four hours
30,000	0.1 c.c.	400	2	0
100,000	0.1 c.c.	5,000	1,000	2,000
1,000,000	0.1 c.c.	400,000	2,000,000	20,000,000

Haas found that the circulating blood is not always bactericidal for any given variety of bacteria to the same extent the serum is.

During the testing of the bactericidal power of the serum on different bacteria it was discovered that numerous varieties were not destroyed by the serum alone, but only when exposed to both serum and leukocytes.

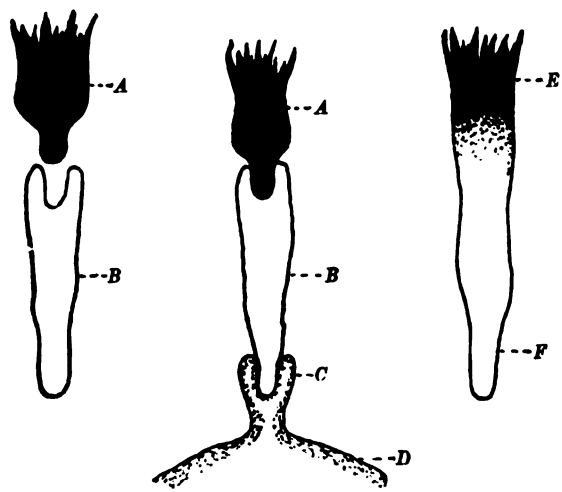
During these earlier years Metchnikoff perceived that the infected host was too little considered, and he drew attention to the rôle of the leukocytes. He noted that in inflammation there is an active migration of leukocytes through the walls of the vessels toward the infecting bacteria. If the bacteria are very virulent they continue to increase, destroying the leukocytes. If the bacteria are not sufficiently virulent to set up a progressive inflammation they are themselves disintegrated. Later it was discovered that bacteria after being acted upon by the serum from the body after infection were much more susceptible to the leukocytes. (See chapter on Opsonins.)

Buchner made many experiments on the nature of the process. He showed that bacteria absorbed these bactericidal substances. Later, Bordet, Ehrlich, and others established that the alexin of Buchner was really a mixture of two types of substances of which one, named "immune body," "sensitizer," or "opsonin" is developed as the result of the injection of foreign cell substance, and the other, named "complement" or "alexin," is present in the blood of normal animals, and is not increased by injection. Neither one of these types of substances alone destroys bacteria, while together they destroy certain varieties. Other bacteria require the action of the complement-like ferment in the leukocytes also.

During the investigations on the bactericidal power of the blood the discovery of the antitoxins which combine with the toxins, but leave untouched the bacteria, was made by Behring and Kitasato, and the nature of the union was investigated by Bordet, Ehrlich, and others. The facts developed by these studies became the basis for Ehrlich's side chain theory.

Ehrlich's Theories Upon Antitoxin Production.—Ehrlich began by observing that of the many poisonous substances known to us only a comparatively small number existed against which we could truly immunize *i. e.*, obtain specific antibodies in the blood serum of the immunized organism. Let us look at two poisons which are very similar in their physiologic action, for example, strychnine and tetanus poison, both of which excite spasms through the central nervous system. One, strychnine, produces no antibody whatever in the serum, while the injection of the other, the tetanus poison, causes the formation of the specific tetanus antitoxin. Ehrlich says that this is because these substances enter into entirely different relations with the cells of the living organism. The one substance, strychnine,

FIG. 65



Graphic representation of receptors of the first and third orders and of complement as conceived by Ehrlich: *A*, complement; *B*, intermediary or immune body; *C*, cell receptor; *D*, part of cell; *E*, toxophorous group of toxin; *F*, haptophorous group.

merely enters into a loose combination with the cells of the central nervous system, so that it can again be abstracted from these cells by all kinds of solvents—*e. g.*, by shaking with ether or chloroform. The combination, therefore, is a kind of solid solution, such as has been shown in the staining with aniline dyes. The tetanus poison, on the contrary, Ehrlich says, is firmly bound to the cell; it enters the cell itself, becoming a chemical part of the same, so that it cannot again be abstracted from the cell by solvent agents. Ehrlich says that the first requirement for every substance against which we can obtain a specific serum must be its power to enter into such a combination with one or more types of cells in the living animal. The substance must possess a definite chemical affinity for certain parts of the organism. Hence, in each substance against which we can specifically immunize, Ehrlich assumes a group of atoms which effects the specific binding to certain cells, the *haptophore group* (Fig.

65, F). Corresponding to this is a group in the cell of the living organism *C*, the *receptor group*, with which the haptophore group combines. The haptophore group is distinct from that part of the substance which exerts the physiologic or pathologic effect, in toxins, for example, from the group which is the carrier of the poisonous action, the so-called *toxophore group E*, or in ferments, from the group which exerts the ferment action, the *zymophore group*. Both groups, haptophore and functional, are independent of each other, and their separate presence can easily be demonstrated because the functional group—*e. g.*, in poisonous toxins the toxophore group —is more readily destroyed by heat than the haptophore group. Thus by heating a toxin for some time to 60° to 65° C. a product will be obtained which is much less poisonous, but which still possesses largely its power to bind antitoxins. In the case of toxins such substances are called *toxoids*. Ehrlich conceived the finer mechanism of the formation of specific substances to be somewhat as follows: The haptophore group is bound to the receptor of the living organism owing to a specific affinity. As a result of this the receptor is lost to the living organism, disposed of, and a biological law formulated by Weigert now comes into action, the law of supercompensation; that is, the organism seeks to replace this defect, but in doing so, not merely replaces the receptors in question, but, according to Weigert, produces more of them than were previously present. The conditions are somewhat like those seen in the callus after a fracture, in which the organism likewise does not produce just the amount of bone previously present; there is always an overproduction.

In this way, Ehrlich states, such a large number of one type of receptors are produced by certain cells, that these become excessive; they are then thrust off into the blood, and these free receptors circulating in the blood constitute the specific antibodies. Ehrlich therefore believes that the specific antibodies in the serum are nothing else than all receptors for which the substance employed in immunization possesses specific affinity. Hence, the same substance which, so long as it remains in the cell, attracts the toxin and makes it possible for that to exert its poisonous action on it, now when it circulates free in the blood or tissue fluids acts as a protection by satisfying the affinity of the poison's haptophore group while still in the blood, and thus preventing the poison molecule from reaching the cell itself.

In the formation of the specific antibodies we must therefore distinguish three stages (Fig. 66):

The binding of the haptophore group to the receptor (2).

The increased production of the receptors following this binding (3).

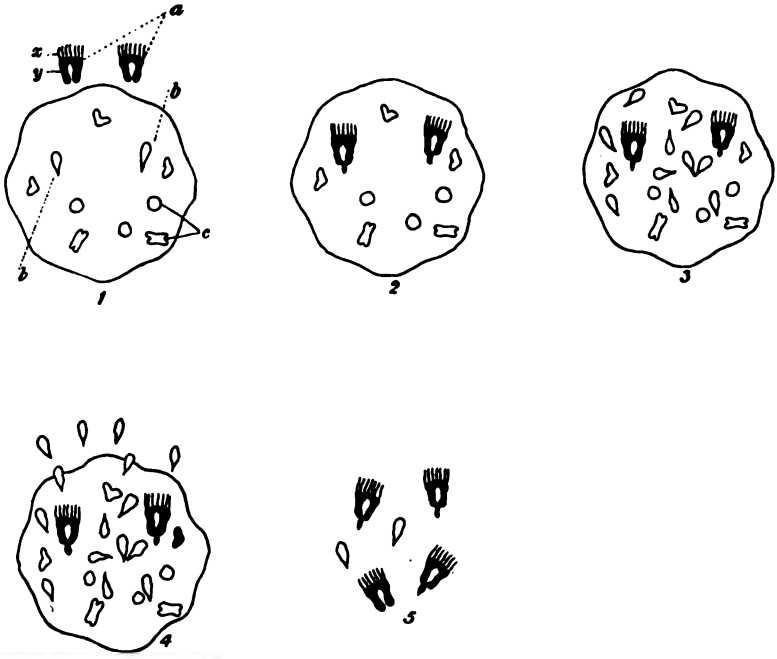
The thrusting off of these increased receptors into the blood (4).

One objection against the Weigert-Ehrlich hypothesis of overproduction of antitoxin by the specifically attacked cells is that while the animals are still showing tetanic symptoms the receptors of the still diseased cells are supposed to have been reproduced, as shown

by antitoxin production. This is answered by Weigert that while the more important cell atom groups are still suffering, the groups producing the receptors may have recovered. This supposition is difficult to prove or disprove.

The idea of Weigert, that the cells are biologically altered so as to continue to make receptors (antitoxin) after the cessation of the injections, and that they increase in capacity to produce antitoxin

FIG. 66



Graphic representation of Ehrlich's theory of the production of antitoxin and the neutralization of toxin: *a*, diphtheria toxin molecule; *x*, toxophore atom group; *y*, haptophore or combining group; *b*, cell receptors with affinity for diphtheria toxin; *c*, other cell receptors.

1. Cell with its receptors. Outside of cell, free toxin molecules.
2. Toxin molecules combined with the cell receptors having affinity for diphtheria toxin.
3. After three days, showing multiplication of cell receptors similar to those combined with toxin.
4. After four days, excess of receptors cast off in the blood.
5. Toxin molecules largely neutralized by combining with free receptors in blood of immunized animal or in animal into which blood with free receptors had been transferred.

as they become accustomed to forming it through the stimulus of repeated injections, is not in accord with the observations made by us. The first point is disproved since there is uniformly a great drop in antitoxin ten days or two weeks after the cessation of the fresh stimulus of renewed injections. The second point is, we believe, rendered improbable by the fact that by partially neutralizing toxin before injecting it into animals, we have found it possible to excite the cells to produce as much antitoxin from the first as from any later injections. An injection into a previously untreated horse of one litre of strong toxin which had been neutralized just sufficient not to poison

a guinea-pig was followed by the development of antitoxin during the following seven days so that each c.c. of serum contained 60 units of antitoxin.

It is true that by the ordinary methods of immunizing the first injections of toxin produce a very small response in antitoxin, but this is because it is possible to give only minute amounts of toxin without causing the death of the animal. Very few cells are thus brought in contact with the toxin.

The Nature of Bacteriolytic, Hæmolytic, Cytolytic Sera.—Bordet, through his own researches and those of Gruber and Durham was able to show that the same type of reaction took place in the animal body when cells of any kind were injected. He showed, for instance, that there was a close similarity between bacteria and the cells of the blood. By immunizing an animal, species *A*, with red blood cells of animal, species *B*, he found that the blood of *A* became hæmolytic for the cells of *B*, just as if immunized with cholera spirilla it would have been bacteriolytic for cholera spirilla. Since then truths obtained from investigation with any type of cells have been applied equally to all others. This allowed the nature of these processes to be studied by Ehrlich, Bordet, and others upon blood cells instead of bacteria.

Experiments Devised by Ehrlich to Show the Nature of Cytolytic (Bacteriolytic, Hæmolytic, etc.) Substances in the Blood.—Ehrlich asked himself two questions: (1) What relation does the hæmolytic serum or its two active components, immune body and complement, bear to the cell to be dissolved? (2) On what does the specificity of this hæmolytic process depend? He made his experiments with a hæmolytic serum that had been derived from a goat treated with the red cells of a sheep. This serum, therefore, was hæmolytic specifically for sheep blood cells—*i. e.*, it possessed increased solvent properties exclusively for sheep blood cells. Ehrlich argued as follows: "If the hæmolysin is able to exert a specific solvent action on sheep blood cells, then either of its two factors, the immune body or the alexin (complement) of normal serum, must possess a specific affinity for these red cells." To show this he devised in conjunction with Morgenroth the following series of experiments:

EXPERIMENT 1.—The serum that was specifically hæmolytic for sheep blood cells was made inactive by heating to 55° C., so that then it contained only the heat resistant substance (immune body). To this was then added a sufficient quantity of sheep red blood cells, and after a time the mixture was centrifuged. Ehrlich and Morgenroth were now able to show that the red cells had combined with all the heat resistant substances, and that the supernatant clear liquid was free from the same. In order to prove that such was the case they proceeded thus: To some of the clear centrifuged fluid they added more sheep red cells; and, in order to reactivate the serum, a sufficient amount of alexin in the form of normal serum was also added. The red cells, however, did not dissolve—there was no sensitizing substance. The next point to prove was that immune body had actually

combined with red cells. The red cells which had been separated by the centrifuge were mixed with a little normal salt solution after freeing them as much as possible from fluid. Then a little alexin in the form of normal serum was added. After remaining thus for two hours at 37° C. these cells had all dissolved.

In this experiment, therefore, the red cells had combined with all the sensitizing substance, entirely freeing the serum of the same.

The second important question solved by these authors was this: What relation does the alexin bear to the red cells? They studied this by means of a series of experiments similar to the preceding.

EXPERIMENT 2.—Sheep red blood cells were mixed with normal—*i. e.*, *not* hæmolytic goat serum. After a time the mixture was centrifuged and the washed red cells tested with the addition of sensitizing substance to determine the presence of alexin. It was found that in this case the red cells, in direct contrast to their behavior toward the sensitizing substance in the first experiment, did not combine with even the smallest portion of alexin, and remained unchanged. This experiment showed that the sensitizing substance first combined with the cell and then only could the alexin unite with the combined cell-immune body complex.

EXPERIMENT 3.—The third series of experiments was undertaken to show what relations existed between the blood cells on the one hand and the sensitizing substance and the alexin on the other, when both were present at the same time, and not, as in the other experiments, when they were present separately. This investigation was complicated by the fact that the specific immune serum very rapidly dissolves the red cells for which it is specific, and that any prolonged contact between the cells and the serum at ordinary temperatures, in order to effect union, is out of the question. Ehrlich and Morgenroth found that at 0° C. no solution of the red cells by the hæmolytic serum takes place. They therefore mixed some of their specific hæmolytic serum with sheep blood cells, and kept this mixture at 0° to 3° C. for several hours. No solution took place. They now centrifuged and tested both the sedimented red cells and the clear supernatant serum. It was found that at the temperature 0° to 3° C. the red cells had combined with all of the sensitizing substance, but had left the alexin practically untouched.

The addition of red cells in the experiments was always in the form of a 5 per cent. mixture or suspension in 0.85 per cent.— *i. e.*, isotonic-salt solution.

The significance of the last of the above-cited experiments is, according to Ehrlich, at once apparent. It is that the sensitizing substance possesses one combining group with an intense affinity (active even at 0° C.) for the red cell, and a second group possessing a weaker affinity (one requiring a higher temperature) for the alexin.

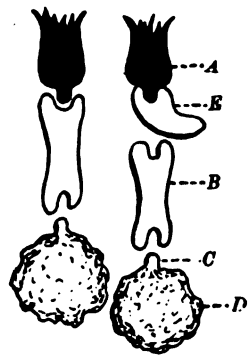
Names Attached to Substances Producing Bacteriolysis.—Different investigators have applied to them different names. The one which is resistant to heat, which attaches itself directly to bacteria,

even at low temperatures, and is increased during immunization, is called sensitizing substance, interbody, amboceptor, or immune body. The other, which is sensitive to heat, which is present in the healthy normal serum, is not increased during immunization, and which unites with the bacterial protoplasm only at temperatures considerably above the freezing point, is called alexin, or complement.

The immune body attaches itself to the bacterial substance, but does not appreciably harm the cells. The complement destroys the cells after the immune body has made the cell vulnerable.

According to Ehrlich, the immune body first unites with the protoplasm of the cell and this develops in the immune body an affinity for the complement and the two unite. (See Fig. 67.) He believes that it is through the immune body that the complement exerts its action on the cell. Very similar to the immune body is the substance called opsonin. This unites with the cell, but instead of making it sensitive to the complement it makes it sensitive to some ferment contained in the leukocytes. The destruction of bacteria by the opsonins and leukocytes will be considered in detail in a special chapter.

FIG. 67



Graphic representation of amboceptor or receptors of the third order and of complement, showing on left the immune body uniting complement to foreign cell and on right the action of anticomplement, binding complement: *A*, complement; *B*, intermediary body; *C*, freceptor; *D*, cell; *E*, anticomplement.

Bordet's Theory.—Bordet supposes that instead of the tissue cell receptors which have combined with the toxin or foreign cell substance (antigen, haptine) producing an excess of similar receptors, that the body of the animal that is immunized instead of reproducing old receptors in large amount without changing them, builds up substances which in their character resemble, but are not identical with pre-existent principles. These new substances have become endowed with a more marked affinity for the specific antigen in question. Bordet considers that Ehrlich in offering explanations which seem definitive has come to make certain problems which have scarcely been touched upon regarded as worked out. According to Bordet, Ehrlich is wrong in attributing such special properties to the immune body rather than at least equally to the antigen. He states that "as a matter of fact, these phenomena should be related, not as regards antigen or antibody considered separately, but as regards the complexes which result from their union, and it is evident that the special properties of the antigen must affect markedly and perhaps to a preponderating degree the qualities of such complexes. Just as the union of agglutinins with bacteria produces in them a remarkable sensitivity to the agglutinating effect of electolytes by modifying their property of molecular adhesion, in a similar way sensitizers confer on their antigens a similar modified property of adhesion, namely, alexin absorption." In his opinion, antibodies, whatever their nature, act very much

alike; but the effects which they produce differ with the antigen in question.

Muir has shown that when cells are saturated with both immune body and complement, the addition of fresh cells causes a splitting off of immune body, but not of complement. This throws further doubt upon the direct union of immune body and complement.

There are exceptional normal sera, the complement of which may be fixed by certain cells without the presence of an immune serum. Malvoz[1] showed that this is the case with dog serum mixed with B. anthracis. This serum acts however as if it contained a true, sensitizer, because in the presence of this organism it will cause the fixation of the complement of the sera of rabbits and guinea-pigs.

Most of the experiments which have been made with the purpose of clearing up these difficult problems have been made upon red blood cells. Here the absorption of the immune bodies at low temperatures and the lack of noticeable injury until the complement is added, at a temperature of 20° to 30° C., is very striking.

Multiplicity of Immune Bodies and Complements.—The immune bodies are very numerous and fairly specific in their action. The complement substance is much less specific and, although probably multiple, when chemically considered each variety acts upon widely different bacteria and cells after they have united with the immune body. There is little reason to think that the complement of one animal is any more capable of attacking bacteria prepared by immune bodies developed in its blood than by immune bodies developed in some other species.

Relation Between Virulence and the Building of Immune Bodies.—It is believed by most to take place the more rapidly the more virulent the infecting organisms. In our experiments this has not been evident. It must be remembered that increase of virulence for one species of animal does not mean increase for all animals; so that in order to draw conclusions, the animal upon which the virulence is tested must be the same variety as the one being immunized.

Origin of Immune Bodies.—Their source must undoubtedly be attributed to the cells, but probably only certain cells produce them. The red blood cells, for instance, seem rather to destroy than to increase them. Injections into the lung and into the subcutaneous tissues of toxins and bacterial substances give rise to the formation of antibodies which are certainly formed partly, if not wholly, locally, and later find their way to the blood. The nuclein derived from the cells, although it has a general bactericidal action, and may enter into the complement (alexin), has different properties, and so cannot itself be one of these bodies.

Origin of Complement (Alexin).—The cells which have abundant nuclear substance, such as the leukocytes and lymph cells, seem especially to be a source, and Metchnikoff asserts their pre-

[1] Annales de l'Institut Pasteur, Aug., 1902.

eminent rôle as the producers of both complements and immune bodies. Buchner and others have found that through the irritation of bacterial filtrates the leukocytes were attracted in great numbers to the region of injection, and that the fluid here, which was rich in leukocytes, was more bactericidal than that of the blood serum elsewhere. Some claim to have demonstrated that along with increased leukocytosis there is a general increase in the complement in the blood; still, it has not yet been positively established that the complement is derived solely from the leukocytes, nor from all leukocytes, and a mere increase in them does not always mean an increase in the complement.

Deflection of the Complement.—It frequently happens that when the addition of a small amount of immune serum renders a normal serum more bactericidal, or an animal immune, a greater addition robs it of most, and sometimes, all of its bactericidal power. This is explained by Neisser and Wechsberg to be due to a locking up of complement by excess of immune body. In Fig. 67 if we substitute an additional immune body molecule (*B*) for the anticomplement (*E*) it would theoretically lock up the complement (*A*) and prevent its union with the immune body which had attached itself to the cell. This is no evidence that amounts of serum even as large as 100 c.c. in an adult, given for therapeutic purposes, have produced deflection of complement. These large injections certainly seem to give the best results. The subject is in need of further study.

Multipartial or Polyvalent Sera.—Bacteria are not homogeneous masses of protoplasm, but are made up of various molecules which differ biologically from one another. Conforming to this, the antisubstances, immune bodies (antitoxins, opsonins, etc.), which appear in a serum are made up of the sum of the antibodies which correspond to these partial elements in the bacterial body. These separate groups are called "partial groups." An immune serum, therefore, consists of the partial groups which correspond to the separate partial elements of the bacterial body. We are further able to show that these partial elements in one and the same bacterial species are not the same for all the bacteria of that species. Thus one culture of streptococci or of *Bacillus coli* may have a few partial elements which differ from those of another culture. What is the consequence of this? The consequence will be that when we immunize with a culture *a* of such bacteria we shall obtain a serum which acts completely on this culture, for in this serum all the partial elements present in culture *a* are represented. If, however, we employ culture *b*, *c*, or *d*, which perhaps possesses other partial elements, we shall find that the serum does not completely affect these cultures. As already stated, such a condition of things is met with in inflammations due to streptococci and other bacteria, and is, therefore, of considerable practical importance. It is because of this fact that a serum from an animal immunized to one culture acts best only in a certain percentage of cases. In order to overcome this

difficulty in persons infected with these bacterial species we have no choice but to make sera, not by means of *one* culture, but by means of a number of different strains of the same species. The result of this will be that, corresponding to the various partial elements in these different cultures, we shall obtain a serum containing a large number of the partial groups. Such a serum will then exert a specific action on a large number of different cultures, but not quite as great an influence on any one as if only that variety had been injected.

In other words, the development and the closer analysis of the problem of immunity, especially during the past few years, have shown us that we must make use, more than heretofore, of so-called *polyvalent* or multipartial sera. In the serum therapy of streptococcus infections, of dysentery, etc., the production of such multipartial sera is an advantage in practice. Owing to these partial groups also, a serum—*e. g.*, anti-typhoid serum—can specifically affect to a very slight degree a closely allied species of bacterium, like *Bacillus coli*, for example. For it is known that closely related species of bacteria possess certain partial groups in common, and a serum is thus produced which to a certain extent acts on such allied species. This constitutes what is known as the "group reaction."

Aggressins.—A further contribution has recently been made to the problems of virulence and immunity in the form of the "aggressin theory" of Bail.[1] Apparently it grew out of an attempt to explain the so-called "phenomenon of Koch"—an observation made years ago by Koch—to the effect that tuberculous animals when inoculated intraperitoneally with a fresh culture of tubercle bacilli succumb quickly to an acute attack of the disease, the resulting exudate containing almost exclusively lymphocytes. Bail found that if tubercle bacilli, together with sterilized tuberculous exudate, were injected into healthy guinea-pigs, the animal died very suddenly—*i. e.*, in twenty-four hours or thereabouts. The exudate alone had no appreciable effect on the animal, while inoculation with tubercle bacilli alone produced death in a number of weeks. He therefore concludes that there is something in the exudate that allows the bacilli to become more aggressive, and hence has called this hypothetical substance "aggressin." He thinks it is an endotoxin liberated from the bacteria as a result of bacteriolysis and that it acts by paralyzing the polynuclear leukocyte, thereby preventing phagocytosis. Heating the exudate to 60° C. increases its aggressive properties rather than diminishes them and small doses act relatively more strongly than larger ones. These facts he explains by assuming the presence of two properties, one that prevents rapid death, is thermolabile and acts feebly in small doses, and one that favors rapid death and is thermostabile. He assumes that in a tuberculous animal the tissues are saturated with the aggressin and when fluid collects in the body cavities, as it does on injection of tubercle

[1] Wiener klin. Woch., 1905, No. 9. Ibid., 1905, Nos. 14, 16, 17. Berliner klin. Woch., 1905, No. 15. Zeit. f. Hyg., 1905, vol. i., No. 3. Arch. f. Hyg., vol. lii., pp. 272 and 411.

bacilli, it contains large quantities of aggressin, which prevents migration of the polynuclear leukocytes, but not of the lymphocytes, and hence allows the bacilli to develop freely, producing acute symptoms. In the peritoneal cavity of the normal animal injected with tubercle bacilli, on the other hand, are large numbers of polynuclear leukocytes which engulf the bacilli, thus inhibiting their rapid development, there being here no aggressin to prevent phagocytosis.

This theory has been applied to a number of infections, including typhoid, cholera, dysentery, chicken cholera, pneumonia, and staphylococcus infections. In all, similar results have been obtained as with tubercle bacilli. When exudates, produced by virulent cultures of these various organisms and properly sterilized, are injected with fresh cultures into an animal, death occurs in much shorter time than when the organisms alone are injected.

Moreover, it has been possible to immunize animals against these various infections by repeated injections of the aggressin in the form of exudates. This results in the formation of an "antiaggressin," which opposes the action of the aggressin, thereby enabling the leukocytes to take up the bacteria and thus to protect the animal. This has been done in staphylococcus, dysentery, typhoid, cholera, pneumococcus, and chicken cholera infection in animals. In addition, a very marked agglutinative property of the blood is acquired for the bacteria in the animals so immunized.

The Fixation of Complement by Sensitized Cells and Its Practical Application.—Bordet[1] and Gengou showed that the existence of a sensitizer, or specific immune body in an antimicrobial serum, by uniting with its specific antigen (bacterium or other cell or proteid material), absorbs alexin (complement).

This experiment of Bordet is usually spoken of as the "Bordet-Gengou phenomenon."

In order to demonstrate this phenomenon of fixation of complement with sensitized antigen (cell protoplasm or soluble proteid), an experiment similar to the following should be made.

Mixtures are prepared into six test-tubes, respectively, as indicated in the accompanying table. The mixture of sensitized blood (sixth column), which is prepared by adding twenty drops of defibrinated rabbit's blood to 2 c.c. of serum from a guinea-pig immunized against rabbit's blood and previously heated to 55° C., is added to each tube after the rest of the mixture has stood at room temperature for about five hours. Hemolysis, which is indicated by the + sign in the table, takes place quickly in tubes 2, 3, 4, because they contain no sensitized antigen to which the complement may become fixed, while in tube 1, which contains such antigen and in tubes 5 and 6 to which no complement serum has been added, no hemolysis occurs.

[1] Annales de l'Institut Pasteur, xv, 1901, 290.

No of tube	Amount of complement serum (fresh normal guinea-pig serum)	Emulsion of antigen	Specific serum heated to 56° C.	Normal horse serum heated to 56° C.	Sensitized blood	Hemolysis
1	0.1 c.c.	0.2 c.c.	0.2 c.c.	.....	0.2 c.c.	—
2	0.1 c.c.	.....	0.2 c.c.	.....	0.2 c.c.	+
3	0.1 c.c.	0.2 c.c.	.....	0.2 c.c.	0.2 c.c.	+
4	0.1 c.c.	.....	.....	0.2 c.c.	0.2 c.c.	+
5	.....	0.2 c.c.	.....	0.2 c.c.	0.2 c.c.	—
6	.....	0.2 c.c.	0.2 c.c.		0.2 c.c.	—

Wasserman[1] and others applied this method in measuring the amboceptor content of specific sera, the most important practical application of its use being in the diagnosis of syphilis. This complicated subject is considered under the subject of syphilis (see under Sec. III).

Hypersusceptibility or Anaphylaxis.—By introducing any strange proteid into the body of an animal, a condition of exaggerated susceptibility to the foreign substance may develop in that animal. This condition is now generally known by the name of Anaphylaxis, a term introduced by Richet (1905) in his studies on the poison of Actiniæ.[2]

The best studied instance of anaphylaxis is that produced in the guinea-pig by the injection of a foreign proteid, such as horse serum, egg white, milk, etc. For example, if a guinea-pig is injected with a small quantity (about 0.01 c.c.) of horse serum, and, after a certain interval (ten to twelve days), is again injected with horse serum, but with a comparatively large amount (3–5 c.c. subcutaneously; 0.25 c.c. intravenously), it will probably die in a short time (within ten minutes symptoms appear in a very sensitive animal and death occurs within an hour). The chief symptoms are respiratory failure, clonic spasms and paralysis. If a smaller dose of serum be given to the sensitized animal it may show only slight symptoms and recover with the production of immunity. This phenominon was noticed simultaneously in several laboratories but was first definitely described by Theobald Smith.

Rosenau and Anderson, who have studied many phases of the subject,[3] found that the young of sensitized animals are also sensitive.

Gay and Southard[4] think that anaphylaxis depends upon the presence of a substance which is contained in the injected proteid and is retained in the sensitized animal. They call this substance anaphylactin.

Tuberculin and malein reactions are well-known instances of anaphylactic manifestations (see special subjects). The so-called *serum sickness* and a further discussion of animal sensitiveness are described in the chapter on diphtheria.

[1] Wassermann, Neisser and Bruck, Deutsche med. Wochenschr., 1906; Wassermann and Plaut, *ibid.*

[2] Richet. Compt. rend. Soc. Biol., 1905, lviii, 109.

[3] Rosenau and Anderson. Jour. Inf. Dis., 1908, v, 85.

[4] Gay and Southard. Journ. Med. Res., 1907 xi, 143.

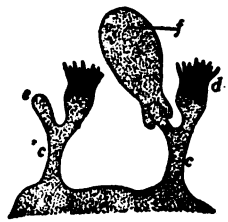
CHAPTER XIII.

THE NATURE OF THE SUBSTANCES CONCERNED IN AGGLUTINATION.

THE discovery of agglutinins in the serum of those passing through many infections was made by Gruber and Durham in 1896, and their characteristics were studied by Bordet. Several months later Widal reported that in typhoid fever the development of agglutinins could be used for diagnostic purposes. Later it was demonstrated that through agglutinins a new means was available for the identification of bacteria. See pages 42–46 for a description of the phenomenon of agglutination and the technique of investigation.

As to the nature of these phenomena a number of theories have been advanced. As in the case of the immune body, there is positive proof that the agglutinin combines directly with substances in the bacterial body. Bordet believes that all the antibodies act very much alike in their method of union and that the effects produced vary with the antigen in question and the characteristics which, on account of its own nature, it can produce as soon as it unites with the appropriate antibody. He does not believe in different families of antibodies, but in an infinite variety of antigens. Ehrlich, on the other hand, considers that the agglutinin consists of a haptophore or combining atom group which is stable and of a ferment group which is labile. The latter causes the phenomenon of agglutination. Bordet draws attention to the fact that bacteria do not agglutinate when they have combined with the agglutinin unless they are in the presence of salt. Agglutinin does not dialyze through animal membranes. In diluted solution agglutinin slowly deteriorates. Dried it lasts longer. It is precipitated with the globulins by ammonium sulphate. When a solution containing agglutinin is passed through a stone filter the first few cubic centimeters contain no agglutinin. The next contain a moderate amount and the remainder the same as the solution.

FIG. 68



Receptors of the Second Order are pictured in *c*. Here *e* represents the haptophore group, and *d* the zymophore group of the receptor, *f* being the food molecule with which this receptor combines. Such receptors are possessed by agglutinins and precipitins. It is to be noted that the zymophore group is an integral part of the receptor.

In some types of infection there is a great accumulation of agglutinins in the blood. Thus in typhoid patients and convalescents distinct agglutination has been observed in dilutions of 1:5000, and this action persisted for months, though not, of course, in the same degree. Even

normal blood serum, when undiluted, often produces agglutination through group agglutinins. But the specific agglutinins, which are formed only in consequence of an infection, are characterized by this, that they produce agglutination even when the serum is highly diluted, and, furthermore, that after this dilution the action is specific—*i. e.*, the high dilutions of cholera immune serum agglutinate only cholera bacilli, of typhoid immune serum only typhoid bacilli, etc. This specificity, however, as will be shown later, is not always absolute.

The agglutinating substances when mixed with bacteria are bound to their agglutinable substances, the two bodies effecting a loose combination very like toxin and antitoxin. By chemical means it is possible again to separate a portion of the agglutinin from bacteria saturated with it and use it to agglutinate bacteria anew.

It was formerly assumed that agglutination was a *prerequisite for bacteriolysis*. This, however, is not so, for both in cholera and in typhoid immunity bacteriolytic substances have been observed without agglutinins, and agglutinating substances without bacteriolysins.

Characteristics of Agglutinins.—Agglutinins changed by heat, acids, and other influences become agglutinoids, which are comparable to toxoids, complementoids, etc.

The union of agglutinin with receptors in bacteria is a chemical or physical reaction, and is quantitative. Before agglutination occurs sodium chloride or a similarly acting compound must be present. The amount of bacteria in the emulsion used to test the amount of agglutinin must, therefore, be known. An emulsion one hundred times as dense as another would require one hundred times as much agglutinin to give an equally complete reaction. Agglutinin acts upon dead bacteria.

Heat diminishes the agglutinability of bacteria when above 60° C. Dreyer found that if a twenty-four-hour bouillon culture of *Bacillus coli* required 1 part of agglutinin to agglutinate it, then if heated to 60° C. it required 2.3 parts; if to 80° C., 18 parts; if to 100° C., 24.6 parts. He found the surprising fact that long heating of the culture restored to some extent its ability to be agglutinated by smaller amounts of agglutinins.

Heated thirteen hours to 100° C., the culture was agglutinated by 4 parts. Dreyer's explanation of this result is that agglutinin-fixing substance is dissolved out by the prolonged heating.

Heating the serum above 60° C. injures the agglutinin slightly, above 70° C. greatly, and above 75° C. destroys it. Weak and strong acids agglutinate bacteria, while medium acidity does not. Alkalies inhibit agglutination. Agglutinin which has lost its power to agglutinate through the effect of heating to 65° C. or through the action of acids usually partially retains its affinity for the bacterial protoplasm. These changed agglutinins are called agglutinoids.

It is important to remember that in low dilutions of serum agglutination may fail, while in higher dilutions of the serum agglutination may take place readily.

The growth of bacteria in fresh blood containing agglutinins inhibits the development of agglutinable substance in bacteria or causes them to produce substances which prevent the union of agglutinin with them. Bacteria should therefore not be grown on serum media when they are to be used in agglutination tests. Even the addition of ascitic fluid to broth has some effect.

Group Agglutination.—Many varieties of bacteria have among the different substances composing their bodies some that are common to other bacteria which are more or less allied to them (Fig. 69). These substances all exciting agglutinins, we have from an immunized animal a serum acting on the different bacteria somewhat in proportion to the amount of protoplasm which they have in common with the infecting organisms. These agglutinins are called, therefore, group agglutinins. If a typhoid or paratyphoid serum possess a high degree of activity—*i. e.*, ability to agglutinate even in large dilution—it may happen that with lesser dilution it may also agglutinate the two related bacilli. Thus, in a case, the infecting paratyphoid bacilli type B were agglutinated 1:5700; typhoid bacilli, however, only 1:120, while paratyphoid bacilli type A were agglutinated only 1:10. In a case of typhoid fever an agglutination of paratyphoid type B occurred with a dilution 1:40, while typhoid bacilli were agglutinated with 1:300. Since it is found that in a paratyphoid infection the serum possesses a fairly strong agglutinating action on typhoid bacilli, Korte advises that in every case of typhoid all three bacteria be tested for agglutination, so that, according to the strongest agglutinating action, one can decide which infection is present. If in practice it is immaterial whether this point be decided, the agglutination with paratyphoid need only be undertaken when the typhoid agglutination is absent.

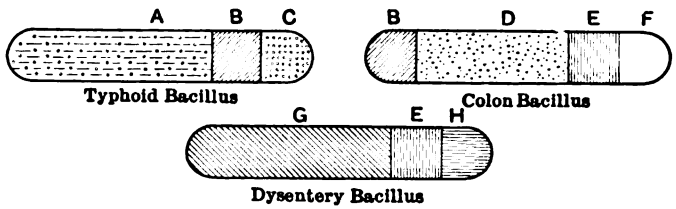
The bacteria which are agglutinated by one and the same serum need not at all be related in their morphological or other biological characteristics, as at first assumed. Conversely, microörganisms which, because of the characteristics mentioned, are regarded as entirely identical or almost so, are sharply differentiated by means of their agglutination. In other words, the "groups" arrived at by means of a common agglutination have no necessary relation to species as the term is usually employed, but only of chemical similarity. This is indicated by the diagrams in Fig. 69. The letters indicate chemical substances capable of stimulating the production of agglutinin and of combining with it when made. Thus both the typhoid and colon will stimulate and react to B agglutinins. Because of this lack of absolute specificity the *diagnosis of the type of infection or the absolute identification of bacteria* through the agglutination or bacteriolytic tests can only be determined with a certain degree of accuracy. This suffices for some infections such as those caused by the typhoid bacillus and the cholera spirillum, but not for others as those due to the colon group of bacilli.

The Development of Agglutinin.—Experimental or natural infection of animals and men is followed in seven to ten days by an appreci-

able development of agglutinin. This development is much greater for some bacteria than for others.

The Relative Development of Specific and Group Agglutinins.—The study of a large number of series of agglutination tests obtained from young goats and rabbits injected chiefly with typhoid, dysentery, paradysentery, paracolon, colon, and hog-cholera cultures has shown that there is considerable uniformity in the development of the specific and group agglutinins. The specific agglutinins develop in larger amount in the beginning, being in the second week usually from five to one hundred times as abundant as the group agglutinins. Later the total amount of the group agglutinins tends to approach more nearly to

FIG. 69



Specific and common agglutinums.

that of the specific, and reach as high at 50 per cent. In a number of tests carried out by us we found that many group agglutinins supplement specific ones in their action, causing by their addition an increased agglutinating strength. In our experience the variety of micoörganism used for inoculation is, if equally sensitive, agglutinated by the combined specific and group agglutinins produced through its stimulus in a higher dilution than any microörganism affected merely by the group agglutinins. It is true that bacteria not injected were at times agglutinated in higher dilutions than the variety injected; this, if not due to greater sensitiveness, was on account of normal group agglutinins present in the animal before immunization. In horses and adult goats it was found that before injections were commenced there was often a great accumulation of agglutinins for bacteria and especially for members of the dysentery, paradysentery, and colon groups, so this comes about through the absorption from the intestines of bacteria of the colon group and the consequent development of agglutinins. For this reason untreated horse serum is a very dangerous substance to use in differentiating the intestinal bacteria. The great height to which the group agglutinins may rise is seen in the following table:

TABLE I.

Agglutinin in the Serum of a Horse Injected with Paradysentery Bacillus. Type, Manila Culture.

Culture.	After 18 injections.			After 21 injections.		
	1:3000	1:5000	1:10,000	1:3000	1:5000	1:10,000
Paradysentery type Manila...	++	−	−	++	++	++
Colon B. X.....	++	++	−	++	++	++

The great amount of agglutinins acting upon the colon bacillus X. is remarkable. A serum is here seen to be acting in dilutions as high as 1:10,000 upon a culture possessing very different characteristics from the one used in the injections.

Although a considerable proportion of the group agglutinins acting on colon bacillus X. was undoubtedly due to the stimulus of the injections of the Flexner paradysentery culture, still a portion of them was probably due to the agglutinins developed by the stimulus of the absorbed intestinal bacteria. In Table II is seen the marked accumulation of agglutinins which may occur in a normal horse before injections are begun.

TABLE II.

	A young horse before inoculation.			
Culture	1:100	1:500	1:1000	1:5000
Dysentery B., Japan	+	−	−	−
Paradysentery, Mt. Desert	+	−	−	−
Paradysentery, Manila	++	++	++	−
Colon B. X	++	+	−	−

The fact of most importance which appears in this table is the abundant agglutinins which may be found in the serum of a horse which has never received bacterial injections.

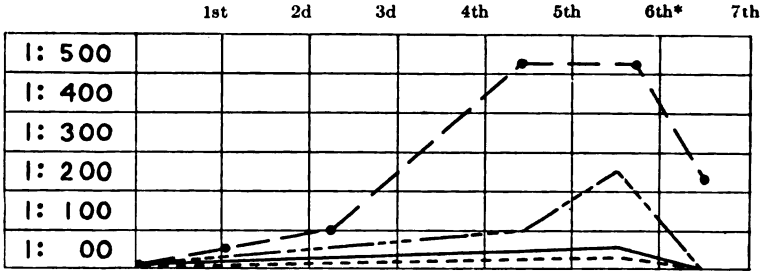
The Relative Accumulation of the Group and Specific Agglutinins for the Organism Injected and for Allied Varieties.—A test was carried out with different types of dysentery bacilli. For the Manila culture of Flexner, which is nearest to the colon in its characteristics, the specific agglutinins were in the serum of an animal which had received injections of the Manilla cultures at the end of the fourth month five times as abundant as the group agglutinin acting on the Mt. Desert culture of Park, which represents a type lying between the Flexner and Shiga-cultures. For the dysentery bacillus (Shiga) the development of agglutinins was the least. (Fig. 70).

Another point of interest is that the proportional amounts of agglutinins from the different cultures varied at different times. If on tests made of a single bleeding we had attempted to draw conclusions as to the relative development of specific and group agglutinins between the cultures, we would have had an imperfect view. Many conflicting statements in literature are undoubtedly due to this lack of appreciation of the variability in the relative amount of these two types of agglutinins during a long process of immunization. (Fig. 71.)

The Use of Absorption Methods for Differentiation between Specific and Group Agglutinins due to Mixed Infection and to a Single Infection.—It is now well established that if an infection is due to one microörganism there will be specific agglutinins for that organism and group agglutinins for that and other more or less allied organisms. If infection is due to two or more varieties of bacteria, there will be specific agglutinins for each of the microörganisms and group agglutinins produced because of each of them.

The following experiments well illustrate these points: A rabbit immunized to *B. typhi* agglutinated *B. typhi* 1:5000, *B, coli* (31) 1:600. After saturation with *B. typhi* all agglutinins were removed for both microörganisms. A rabbit immunized to both *B. typhi* and *B. coli* (31) agglutinated *B. typhi* 1:4000, *B. coli* (31) 1:1000. (After saturation with *B. typhi* the serum did not agglutinate *B. typhi*, but *B.*

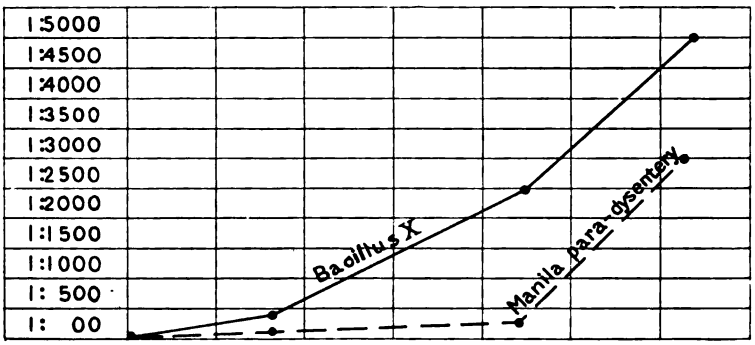
FIG. 70



The rise and fall of common and specific agglutinins during seven months in a rabbit injected with the Manila culture.

————Colon bacillus X.
– – – – – – Paradysentery type (Mt. Desert).
— — — — — Paradysentery type (Manila).
- - - - - - - - - - - Dysentery type (Japan).
•Test dates for all four sera.
*Injections stopped.

FIG. 71



Similar conditions to those noted in previous chart, except that a young goat has been used for the injections of the colon bacillus X. The great accumulation of common agglutinins for the paradysentery bacillus in the third month of the injections of the bacillus X is very striking.
•Tests made.

coli (31) 1:900.) After saturation with *B. coli* (31) it failed to agglutinate *B. coli* (31), but still agglutinated *B. typhi* 1:3500. Some other strains of *B. coli* still agglutinated in 1:20 or more because many strains included in this group act as differently toward each other in respect to agglutinins as they do to the typhoid bacilli.

The following tables give the outcome of several experiments:

The great number of varieties of the colon group of bacilli that are in the normal intestine and which are absorbed slightly in health and

more markedly in intestinal diseases make the use of absorption tests for diagnostic purposes too complicated except for peculiarly important cases and require trained skill to carry them out.

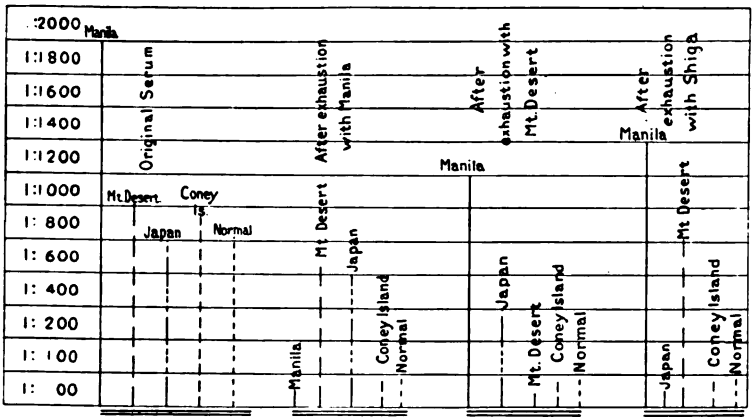
ABSORPTION BY THE TYPHOID BACILLUS OF GROUP AGGLUTININS ACTING UPON A NUMBER OF VARIETIES OF B. COLI WHICH WERE PRODUCED BY ANOTHER VARIETY OF B. COLI.

Agglutination by Serum of Rabbit Immunized to Colon Bacillus X.

	Before addition of typhoid bacilli.	After attempt at absorption with typhoid bacilli at 22° C.
Colon bacillus X	6000	5000
Colon bacillus 1	500	20
Colon bacillus 2	500	30
Colon bacillus 3	250	30
Colon bacillus 4	250	10
Colon bacillus 5	10	less than 10
Colon bacillus 6–18	less than 10	less than 10
Typhoid bacillus	less than 10	less than 10

The absorption tests were carried out by adding the bacilli from recent agar cultures to a 10 per cent. solution of the serum in a twenty-four-hour bouillon culture. The mixture was allowed to stand for twenty-four hours at about 22° C. It was found that the agglutinin in a simple dilution of serum when left at 37° C. rapidly deteriorated. Thus, in an extreme instance a serum positive at 1 : 1500, when diluted with bouillon or salt solution 1: 25 and left at 37° C. for twenty-four hours, lost 30 to 40 per cent. of its strength; at 22° C. it lost at times 15 to 20 per cent. Left for three hours only, the loss was only 5 to 10 per cent.

FIG. 72



Showing the effect of saturating with bacilli of types of Shiga-Manila and Mt. Desert, a serum from a horse which had received combined injections of dysentery bacilli of the three types. Note that the Manila type removed almost all the specific and group agglutinins acting upon its own type and the group agglutinins acting upon the Coney Island and normal types, leaving the specific agglutinins for types Shiga and Mt. Desert. The same is true for types Shiga and Mt. Desert when they were used.

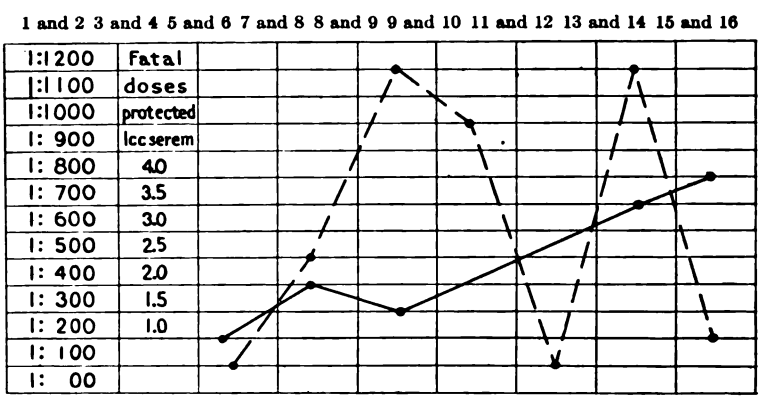
– – – – – – – – Manila paradysentery.
— — — — — — Japan dysentery.
— — — — — — — — Mt. Desert paradysentery.
— — — and — — — Atypical paradysentery.

The absorption method simply proves, therefore, that when one variety of bacteria removes all agglutinins for a second the agglutinins under question were not produced by that second variety.

Loss of Capacity in Bacteria to be Agglutinated or to Absorb Agglutinins Because of Growth in Immune Sera.—The loss of these characteristics by growth in sera has been demonstrated by Marshall and Knox. The experiments of Collins and myself are recorded because they were undertaken in a slightly different way and also because a certain number of confirmatory observations are of value.

The maltose fermenting paradysentery bacillus of Flexner was grown on each of eleven consecutive days in fresh bouillon solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The solutions used were 1.5, 4, and 15 per cent. The serum agglutinated the culture before its treatment in dilutions up to 1 : 800, and was strongly bactericidal in animals. After the eleven transfers the culture grown in the 15 per cent. solution ceased to be agglutinated by the serum and ceased to absorb its specific agglutinins. The cultures grown in the 1.5 and 4 per cent. solutions agglutinated well in dilutions up to 1: 60 and 1: 100 and continued to absorb agglutinins. The recovery of the capacity to be agglutinated was very slow when the culture was from time to time transplanted on nutrient agar. After growth for sixteen weeks, during which it was transplanted forty-three times, it agglutinated in dilutions of 1 : 200. The culture grown in 4 per cent. agglutinated 1 : 500, and the one

FIG. 73



Relation of agglutinative power to bactericidal. Horse injected with Manila culture over a period of sixteen months.

— — — — — Agglutination index.

———— Bactericidal index.

• Test dates.

in 1.5 per cent. 1: 800. This diminution and final cessation of development of agglutinable substance in bacteria grown in a serum rich in agglutinin and immune bodies is interesting both as showing the variation of the bacteria and as one means of adapting themselves to resist destruction, since the bacteria which ceased to produce agglutinable substance probably also produced less substance with affinity for other antibodies. This inhibition of the production of agglutinable

substance was also very noteworthy in the case of pneumococci grown in serum media.

Relation between Agglutinating and Bactericidal Power.—In spite of proof to the contrary good observers hold to the belief that there is some relation between the agglutinating and the bactericidal strength of a serum. The tests we carried out on the serum of a number of horses showed no such relation. In Fig. 73 are recorded a number of comparative tests during a period of sixteen months. The tests of the bactericidal power of the serum were made by Goodwin.

Variation in the Agglutinating Strength of a Serum.—There is usually a continued increase in the amount of agglutinin in the blood of an infected person from the fourth day until convalescence and then a decrease. At times, however, there is a marked variation from day to day, so that it may be abundantly present one day and almost absent the next.

Precipitin.—A substance similar to, but altogether distinct from, agglutinin is precipitin. This substance was discovered by Kraus in 1897. He found that when a little immune serum was added to the bacteria-free filtrate of a culture of the organism used to produce the immunization there occurred a precipitate. This same reaction took place between the serum of an animal injected with various proteid substances, such as white of egg, blood serum, milk, etc. Precipitins in their development, their resistance to heat and chemicals and in their specific and non-specific forms are similar to agglutinins. The precipitins have been used more in relation to blood identification than in bacteriology. The specificity of precipitins is, like that of the agglutinins, not absolute. Group precipitins act upon similar chemical substances derived from cells having very different characteristics. The precipitin test is mostly employed in testing sera and tissue extracts rather than bacterial filtrates.

As the action of bacterial precipitins seems to be parallel with the action of the agglutinins, it is not possible that where tube reactions are depended upon, some confusion may occur as to which substance is really affected by certain processes or agents, especially those having a solvent action upon the bodies of the bacteria.

CHAPTER XIV.

OPSONINS[1]—EXTRACT OF LEUKOCYTES—BACTERIAL VACCINES.

THE original theory of Metchnikoff, that the leukocytes were the only actual protective bodies which warded off disease, and that they did this by attacking the bacteria, was founded on the now well-known fact that certain of the white cells possess the power of taking up into themselves pathogenic bacteria, and that they are there destroyed. It was later observed that these cells have the property of taking from the blood many lifeless foreign elements, thereby keeping the blood channels free of foreign particles.

The question thereby arose as to whether these cells engulfed and then killed the bacteria, or whether perhaps other substances killed or prepared them before the cells took them up. It became known that certain bacteria are killed solely by the bactericidal substances in the serum, while others are not killed until taken up by the leukocytes. The leukocytes and the chemical substances of the blood thus both play an important part. The death of the bacteria also liberates positive chemotactic substances, and the disintegration of the white blood cells gives rise to bactericidal bodies. We find that phagocytosis is most marked when the disease is on the decline or the infection mild, but is usually absent in rapidly increasing infection. This would seem to indicate that the course of the infection is often already determined before the leukocytes become massed at the point of its entrance. The first determining influence is given by the condition of the tissues and the amount of bactericidal substances contained in them, and then, later, in cases where the bacteria have been checked, comes the additional help of the leukocytes. If the tissues are wholly free of bactericidal and sensitizing substances, neither they nor the leukocytes, nor both combined, can prevent the bacterial increase. The simple absorption by the cells of bacteria is not necessarily a destructive process. Metchnikoff believes that the polymorphonuclear leukocytes are especially antibacterial in relation to acute infections. The large phagocytes are conceived to deal chiefly with the resorption of tissue cells and with immunity to certain chronic diseases, such as tuberculosis.

The present great interest in the subject of the opsonins is largely due to the investigations and influence of Wright. We should, however, recognize the important earlier work of others. Denys and

[1] Greek "opsono"—I cater for.

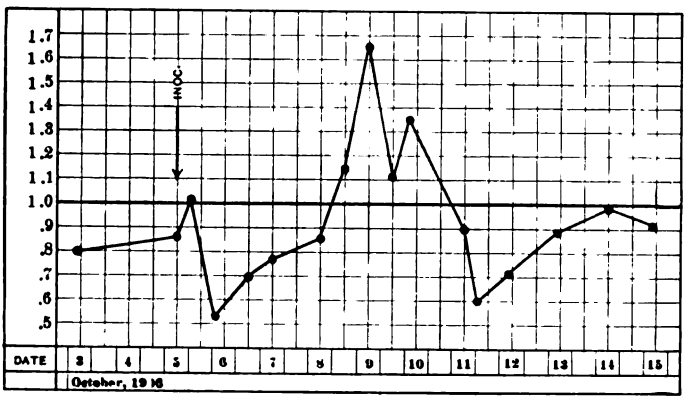
Leclef had previously shown that in the case of rabbits immunized against streptococci, the increased phagocytosis was due to an alteration in the serum and not to changes induced in the leukocytes. They demonstrated that the leukocytes of the immunized animal when placed in normal serum showed no greater phagocytic activity than normal leukocytes did. Wright added the important fact that the substances in the serum favoring phagocytosis united with the bacteria. Neufeld and Rimpau discovered the same point independently. Wright dealt mostly with normal serum, while Neufeld used serum from immunized animals.

Wright originated the idea of estimating the changes in the opsonic power of the blood for the purpose of guiding the use of vaccines in the treatment of bacterial infections. Thus he states:

"I have found that there exists in the serum of the successfully inoculated patient an increase of opsonin. This is a substance which lends itself to very accurate measurement by a modification of Leishman's method. By the aid of this method the patient's progress or regress can be very accurately followed."

Where vaccines are injected Wright states there "supervenes a negative phase where there is a diminished content in protective substances. This is succeeded by a positive phase. This inflowing wave of protective substances

FIG. 74



An opsonic curve showing the slight immediate rise and the later negative and positive phases following inoculation. The changes here are more regular than generally occurs.

rapidly flows out again, but leaves behind in the blood a more or less permanently increased content of protective substances. When a small dose of vaccine is given the negative phase may hardly appear, but the positive phase may be correspondingly diminished. Where an unduly large dose of vaccine is inoculated the negative phase is prolonged and much attenuated. The positive phase may in such a case make default."

"It will be obvious that, if we, in the case of a patient who is already the subject of a bacterial invasion, produce by the injection of an excessive dose of vaccine a prolonged and well-marked negative phase, we may, instead of

benefiting the patient, bring about conditions which will enable the bacteria to run riot in his system."

"Now, consideration will show that we may obtain, according as we choose our time and our dose wisely or unwisely, either a cumulative effect in the direction of a positive phase or a cumulative effect in the direction of a negative phase. We may, in other words, by the agency of two or more successive inoculations, raise the patient by successive steps to a higher level of immunity, or, as the case may be, bring down by successive steps to a lower level. We can select the appropriate time and dose with certainty only by examining the blood and measuring its content in protective substances in each case before reinoculating."

These statements of Wright have exerted a great influence, for, if he is correct, it will be desirable for every city to have laboratories equipped not only for supplying vaccines, but also for determining the opsonic index in cases suitable for inoculation. Wright claims two fundamental points—first, that it is possible to determine the real opsonic power of the blood with sufficient accuracy to make it available for treatment, and second, that the opsonins are either the most important of the protective substances of the blood or that they undergo a sufficient proportional development with the latter to be a safe guide as to their amount. A knowledge of the opsonic content of the blood is also believed by him to give information as to the presence and gravity of an infection.

An immense amount of investigation has revealed the fact that the index cannot be obtained accurately enough to be a safe guide in single tests to be used in diagnosis or treatment unless the variation from the normal is exceptionally great and that the opsonic content is not a safe guide for the measure of the total antibodies in the blood.

THE OPSONIC INDEX.

Technique.—Wright's technique of measuring the opsonic power is a slight modification of the Leishman[1] method and is as follows: An emulsion of fresh human leukocytes is made by dropping twenty drops of blood from a finger prick into 20 c.c. normal salt solution containing 1 per cent. sodium citrate. The mixture is centrifuged, the supernatant clear fluid removed and the upper layers of the sedimented blood cells transferred by means of a fine pipette to 10 c.c. normal salt solution. After centrifuging this second mixture the supernatant fluid is pipetted off and the remaining suspension used for the opsonic tests. Such a "leukocyte emulsion," of course, contains a mixture of leukocytes and of red blood cells; the proportion of leukocytes, however, is much greater than in the original blood. The bacterial emulsion is prepared by gently rubbing a little of the culture to be tested in salt solution (0.85 to 1.2 per cent.). When thoroughly mixed the fluid is centrifuged for a few minutes so as to remove any clumps. The emulsion should be so thick that in a trial test the leukocytes take up about five apiece on the average.

[1] Leishman, British Medical Journal, Jan., 1902.

One volume of the leukocytes is mixed with one volume of the bacterial suspension to be tested and with one volume of the serum. This is best accomplished by means of a pipette whose end has been drawn out into a capillary tube several inches in length. With a mark made about three-quarters of an inch from the end it is easy to suck up one such volume of each of the fluids, allowing a small air bubble to intervene between each volume. All three are now expelled on a slide and thoroughly mixed by drawing back and forth into the pipette. Then the mixture is sucked into the pipette, the end sealed, and the whole put into the incubator at 37° C. The identical test is made using a normal serum in place of the serum to be tested. Both tubes are allowed to incubate fifteen minutes and then the end of the tube is broken off, a large drop mounted on a clean slide the surface of which was previously roughened by emery-paper and a spread made with a second slide as in ordinary blood work, only a little thicker and using no force whatever. After drying in the air the smears are stained without previous fixation either with a 1 per cent. aqueous solution of methylene blue or some other suitable stain. The degree of phagocytosis is then determined in each by counting a consecutive series of fifty or one hundred leukocytes and finding the average number of bacteria ingested per leukocyte. This number for the serum to be tested is divided by the number obtained with the normal serum and the result regarded as the *opsonic index* of the serum in question. Thus, if the tubercle bacilli, sensitized by a patient's blood, are taken up by the leukocytes to the average number of three per leukocytes, and bacilli from the same emulsion sensitized by normal blood are taken up by leukocytes to the average of five, then the index will be three-fifths of one, or 0.6. In this case the index would indicate a deficiency in opsonins. The presence of a high opsonic index Wright regards as indicative of increased resistance. He further states that the fluctuation of the opsonic index in normal healthy individuals is not more than from 0.8 to 1.2, and that an index below 0.8 is, therefore, almost diagnostic of the presence of an infection with the organism tested.

Simon's Method: Simon has suggested a modification of Wright's method. He estimates the percentage of phagocyting cells in the mixture containing the serum to be tested and compares this with the mixtures containing normal serum. He also suggests that dilutions of blood be tested.

The Dilution or Extinction Method recommended by Dean and by Klein. The degree of dilution of the serum necessary for the extinction of its opsonic index is determined; that is, the serum to be tested is diluted until a dilution is found which shows the same small amount of phagocytosis shown in preparations in which no serum is used, namely an index below 0.5. Klein claims that results by this method are more accurate than by the method of Wright. The method is too tedious for practical use in routine work.

Most workers are now agreed that the use of the opsonic index is limited to experimental investigations.

THE ACCURACY WITH WHICH THE OPSONIC POWER OF THE BLOOD CAN BE DETERMINED BY WRIGHT'S METHOD.—An examination of any slide will show that the different leukocytes vary in their size and in their content of bacteria. This is due partly to variation in phagocytic activity, and partly to the interference of the red blood cells, which are present in great numbers in the emulsion and separate the bacteria in different degrees from the white cells. These and other reasons bring it about that the different leukocytes vary greatly in the number of bacteria they take up and in their distribution on the slide. Partly to overcome this, large numbers of leukocytes are counted. Beyond one hundred, or at most one hundred and fifty, the increase of accuracy hardly compensates for the extra labor. The following table shows the difference between counting larger or smaller numbers of cells in five opsonic tests as determined by counting different numbers of cells in one specimen.

OPSONIC INDEX ESTIMATIONS IN FIVE BLOOD SPECIMENS.

Cells counted	Average number of bacteria in each leukocyte.				
50	1.18	1.88	1.34	1.42	1.90
100	1.22	1.78	1.24	1.42	1.59
150	1.18	1.62	1.22	1.44	1.50
200	1.18	1.51	1.22	1.46	1.37
600	1.28	1.62	1.23	1.36	1.36
1,200	1.34	1.44	1.25	1.30	1.42

It is noticed that the variation between the average cell count obtained from fifty cells and larger numbers is much greater than between that obtained at from one hundred or one hundred and fifty.

It is necessary to have the counts that are compared all counted by the same person, as each individual has a somewhat different method and will average higher or lower for all counts than any other person.

When two specimens of blood are tested not only the inaccuracy of counting due to the different arrangement of the unequally filled cells on the slides to be counted is met, but the fact that in making the test the conditions are not similar, for in different mixtures slightly different proportions of leukocytes, bacteria, and red cells will always be mixed together. If smears from a series of tubes of the same blood are compared with a series of smears from one of the tubes, the former will always show the greater variation.

This variation is much greater than most examiners believe. North has collected a series of tests carried out in nearly all the important laboratories in the Eastern United States that are working upon opsonins. The results recorded prove absolutely that while an average counting error of only about ten per cent. is present, there may be an exceptional error of at least 100 per cent., and one of at least

20 per cent. may be expected once in about every ten determinations.

The following is a fair average of the correctness of routine tests by experienced workers.

ABSOLUTE COUNT OF BACTERIA IN ONE HUNDRED LEUKOCYTES.

Blood specimen A.		Blood specimen B.		Blood specimen C.	
Tube 1.....	156	Tube 1.....	142	Tube 1.....	89
Tube 2.....	168	Tube 2.....	182	Tube 2.....	102
Tube 3.....	172	Tube 3.....	188	Tube 3.....	121
Tube 4.....	198				

This error, which occurs because of the technique, applies not only to the examination of the specimen of blood, but also to the measure we employ to estimate the amount of opsonins. As these are not stable, we cannot have a standardized solution, as we do with antitoxins. We must, therefore, determine our measure afresh in each test, taking for this purpose a supposedly normal blood. Wright, from a great many tests, has determined that the opsonic power of the blood in non-infected persons for tubercle bacilli does not vary, as a rule, more than 10 per cent. above or below the average power of healthy blood. For staphylococci there is more variation. It is found also that many things besides infection decrease the amount of opsonins in the blood. Hemorrhage, fatigue, starvation, and other influences which lower the resistance of the body have this effect.

Wright gets this measure as uniform as possible by determining the average opsonic strength of five supposedly healthy persons at the time of each test. If any one of these five is considerably below or above others it is omitted for that day. The measure so obtained will probably vary above 5 per cent. from day to day, though seldom getting far away from what we might call the absolute normal. The following results were obtained by us from examining at one test a number of supposedly normal persons against tubercle bacilli and staphylococci.

OPSONIC COUNTS IN TEST OF TWENTY-ONE NORMAL SERA WITH STOCK STAPHYLOCOCCUS CULTURE.

1.....	4.13	8.....	3.82	15.....	9.09
2.....	2.93	9.....	3.95	16.....	5.17
3.....	2.78	10.....	3.98	17.....	4.04
4.....	4.37	11.....	4.27	18.....	3.82
5.....	3.58	12.....	3.69	19.....	4.00
6.....	2.90	13.....	3.80	20.....	3.79
7.....	3.56	14.....	3.59	21.....	3.44

OPSONIC COUNTS IN EIGHTEEN CONSECUTIVE NORMAL CASES WITH TUBERCLE BACILLI.

Case	Average number bacilli	Opsonic index	Case	Average number bacilli	Opsonic index
1	2.46	1.00	10	2.05	.83
2	3.20	1.30	11	2.21	.90
3	2.90	1.14	12	2.86	1.17
4	2.66	1.08	13	2.81	1.14
5	2.75	1.12	14	2.79	1.14
6	2.30	.94	15	3.34	1.32
7	2.40	.98	16	2.96	1.02
8	1.88	.76	17	2.16	.88
9	1.73	.70	18	3.12	1.27

THE INFLUENCE UPON THE OPSONIC TEST OF THE SPECIFIC DIFFERENCES BETWEEN STRAINS OF A SINGLE SPECIES.—The general practice in laboratories is to use stock cultures of tubercle bacilli, staphylococci, and other bacteria for the opsonic tests. To obtain a culture from a case may be at first impossible and, if successful, causes a delay of at least one or two days. The culture when obtained may also, as is frequently the case with pneumococci and streptococci, fail readily to opsonize.

These and other reasons tend to establish the use of laboratory stock cultures, and yet we must acknowledge that when we test the amount of opsonins by both the stock and fresh cultures a marked difference sometimes develops. This factor of individual specificity must therefore be taken into account in our interpretation of the accuracy of an opsonic test.

THE LEUKOCYTES TO BE EMPLOYED.—To many it seems a matter of indifference whether one person's leukocytes or another's are used, but our experience agrees with that of others that the leukocytes from different persons not only vary in their activity, but also in their selective action, and that the index is not the same when obtained with one person's leukocytes as with another's.

THE INFLUENCE OF THE STRENGTH OF THE BACTERIAL EMULSION.—The more abundant the bacteria the greater will be the number taken up by the leukocytes. It is very important therefore that the tests be made with the same strength of emulsion.

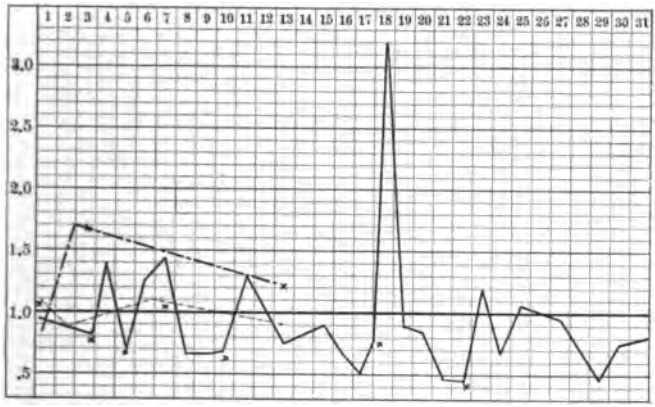
THE OPSONIC VARIATION DURING TREATMENT BY INOCULATIONS.—Wright lays stress on the considerable uniformity of the degree and persistence of development of opsonins after inoculation. We have found in a small percentage of cases typical increases and decreases, as seen in Fig. 74, but in the majority of those inoculated there has been great irregularity. Frequently the negative phase does not occur or at least it is not detected.

The following chart for three staphylococcus cases illustrates this (Fig. 75):

THE VARIATION IN THE AMOUNT OF OPSONINS IN SUPPOSEDLY HEALTHY PERSONS.—It has already been noted that in getting our

measure we test a number of persons and exclude the blood of those which varies greatly from the average. We are so in the habit of seeing the normal blood placed at unity because it is each day the measure of comparison and therefore is one that even investigators are

FIG. 75



x = injection of vaccine. 3 types of lines = 3 cases.

apt to think of the indices of normal persons as being unchanged from day to day. This is not the fact. A glance at the next chart, in which three cases of tuberculosis are charted together with two normal persons, shows that the variation is only slightly greater in infected

FIG. 76

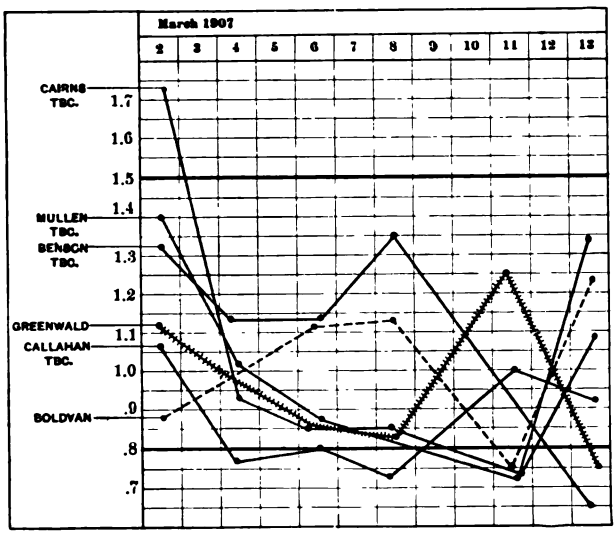


CHART 2.

Dotted and crossed lines, normal persons. Continuous lines, tuberculosis.

than in normal cases. If one normal person is charted against another for several weeks, marked differences will usually appear. The indices of the twenty-one normal cases tested against staphylococci and the eighteen against tubercle bacilli (pages 177 and 178), illustrate this variation in the amount of opsonins in normal blood.

THE OPSONIC INDEX CANNOT BE KNOWN AT THE TIME THE TREATMENT IS GIVEN.—Most of those who have not carried out inoculations under the guide of the opsonic tests think that the vaccinator is guided at the moment of injection by his knowledge of the opsonic power of the blood at the time. A moment's thought reveals that this is an absolute impossibility. In fact, except under very unusual conditions, it is impossible to have the test of the opsonic power reported within twenty-four hours, and in the treatment of the poor in out-patient practice longer intervals usually elapse, so that the treatment is given on a test made either the day before or, more often, on from three to seven days before. As can be seen by the three curves on page 179, which are quite as uniform as the average, it is impossible to judge what the index is at any moment by looking at the indices of blood taken from one to seven days previously.

Other methods have been devised to get more accurate information upon the opsonic contents of the blood. The dilution method and that combined with the determining the percentage of phagocytes absorbing bacteria are the most valuable[1] (p. 175). For experimental work they have advantages, but for practical use in governing the dosage of vaccines they have most of the drawbacks of Wright's method.

THE NATURE OF OPSONINS.—Wright and Neufeld, in their original experiments differed as to the effect of heat on opsonins. Further investigation has shown that opsonins in those not immunized are largely thermo-labile, while opsonins developed after immunization are resistant. Muir and Martin believe from their experiments that the thermo-labile opsonin of normal serum and the thermo-stable opsonin are two entirely distinct classes of substances. The thermo-stable substance is of the nature of a true antibody and possesses the comparatively specific qualities of antibodies in general. Powerful complement absorbers have no effect on the thermo-stable opsonin, but do remove almost completely the thermo-labile opsonin.

Emulsions of other than the organisms used in immunization do not absorb a large percentage of the immune opsonin, but do of the complement opsonin.

We have carried out absorption experiments with staphylococci, colon, and tubercle bacilli. Our results were similar to those of Muir and Martin.

OPSONIN DEFICIENT IN CEREBROSPINAL FLUID AND IN EXUDATES.—Opie[2] has shown that exudates produced by injecting microörganisms usually have little or no opsonin for the variety injected or for other varieties. Hektoen has showed that opsonins, like other anti-

[1] Simon, Jour. Exp. Medicine, Vol. 9, No. 5, 1907, page 487.
[2] Opie, Jour. Exp. Med., Vol. 9, No. 5, p. 515.

bodies, are almost absent in the spinal fluid. McKenzie and Martin[1] showed that in a case of cerebrospinal meningitis the spinal fluid showed no immune bodies while the blood contained them in abundance.

COMPARISON BETWEEN OPSONINS AND BACTERICIDAL SUBSTANCE IN THE SERUM.—We have made comparative tests between the opsonic and bactericidal power of the cell-free serum in typhoid infection and found that they did not run parallel. The frequent rapid increase in opsonic power within twelve hours of an injection of bacteria is striking and very different from the development of bactericidal strength.

Results Obtained by Vaccine Therapy.—In most cases, the employment of vaccine therapy directed to the destruction of a single species of microbe leaves the other species quite unaffected. When in cases of mixed infection measures are taken to immunize the patient against each of the different infections, the task of the immunizator is more laborious and more intricate. On the other hand, the organism of the patient does not seem to find the task of responding to a series of different vaccines (always supposing that each of these is administered in appropriate and properly interspaced doses) much more difficult than the task of responding to one variety of vaccine only.

Although during the past three years many thousands of cases of different types of bacterial infection have been treated by vaccines, there is at present considerable difference of opinion as to their value. The majority of observers agree that it is in subacute and chronic infections that vaccines give the best results. Thus a case of acute streptococcus septicæmia, which after a week or ten days shows a tendency to abate with localization in a joint or in a valve of the heart, offers a much better chance for vaccine treatment than such a case during the early more acute stage. Pneumonia, which after partial recovery persists, a gonorrhœal joint, a persistent pus sinus, a localized inflammation due to colon bacilli, are all considered suitable for treatment. The use of vaccines in cases of acute inflammation of the mucous membranes of the intestines, bladder, throat, etc., have in most hands given rather negative results. Most believe that the inflammation is lessened, but as a rule the bacterial infection, though it becomes latent, still remains and a relapse may occur later.

The different bacteria seem to differ in the successes with which they are used as vaccines. Nearly all observers report the greatest success with staphylococcus infections. These not only when they are severe as a subacute septicæmia or a very severe carbuncle, but also when they are mild, such as ordinary furuncles or acne, seem to be aided by the vaccine treatment. Gonorrhœal joints seem to respond well, while acute gonorrhœa of the mucous membranes responds less readily. Suitable cases of inflammations due to Friedlander's bacillus, to the micrococcus catarrhalis, and many other varieties respond quite well to inoculations.

[1] Jour. of Path. and Bact., Vol. xii, p. 539.

The streptococcus infections seem to be more resistant, and, although in the something like forty cases of subacute septicæmia due to these organisms which have been treated the results indicate some benefit, the effects of the vaccines are not well-established. The pneumococcus also seems resistant, perhaps even more so than the streptococcus. Numerous cases of pneumonia have been treated with somewhat doubtful results. Some observers treat these pneumococcus and streptococcus inflammations with daily doses of 5 million instead of the large semi-weekly inoculations. Various sinus infections of the head have been treated, but with doubtful results. Streptococcus and other inflammations of the gums and teeth have yielded apparently good results. The treatment of tuberculosis with the different tuberculins has given perhaps the most extended use of vaccines. The majority of observers are convinced that in cases of tuberculosis, in which the symptoms have become quiescent, whether they are incipient or fairly advanced, that tuberculin injections do good. Some believe in giving very minute doses, and only slightly increasing their size, others believe that different cases will receive with benefit different amounts, and so try to detect which will stand larger amounts. Nearly all physicians try to prevent definite reactions. The treatment of tuberculosis is considered in detail under tuberculosis.

Preparation of Vaccines.—Bacterial vaccines heated above 60° show marked changes in their chemical composition, and do not yield the same amount of antibodies as those not so heated. Even lower degrees, such as 56°, are preferable to 60°. It has lately been suggested that the vaccines be killed by ½ per cent. carbolic acid, or ¼ per cent. lysol instead of by heating. These vaccines seem to be somewhat more capable of giving good response and they preserve their characteristics for a longer period. Such vaccines can be kept for a period of six months to one year.

AMOUNT OF VACCINE INJECTED.—Different observers advise that for each organism the following number of *millions* be given:

	Minimum.	Maximum.	Average.
Staphylococcus	50.0 m.	1000.0 m.	250.0 m.
Streptococcus	2.5 m.	100.0 m.	25.0 m.
Pneumococcus	2.5 m.	100.0 m.	25.0 m.
Gonococcus	2.5 m.	300.0 m.	30.0 m.
B. coli group	5.0 m.	1000.0 m.	100.0 m.
B. pyocyaneus	5.0 m.	1000.0 m.	100.0 m.
Tuberculin (B. E.)	.00003 mg.	.0005 mg.	.0003 mg.

The injections are usually given at intervals of five to ten days but sometimes daily.

Sensitized Vaccines.—Besredka[1] has suggested a plan of injecting virus which has been immersed in specific serum; in other words, of using virus which has been sensitized. Heated vaccines alone sometimes give severe symptoms when inoculated, *e.g.*, typhoid, cholera; but after exposure to serum, inoculations of the sensitized vaccines are

[1] Review of previous work. Bull. Inst. Pasteur, 1910, viii, 241.

found to give practically no negative phase and only slight local and general reactions. Immunity, moreover, is produced rapidly by this procedure. Such sensitized vaccines can be given in doses thirtyfold those of pure virus. Marie[1] has used the same method in rabies treatment. We have only used sensitized vaccines in animal experiments and have not found as good a response as to the corticated bacteria.

The method is as follows: (1) *For bacteria.*—A twenty-four-hour agar culture of bacteria is mixed with specific serum at room temperature and allowed to stand for three hours. The bacteria are then washed free from unattached serum by repeated centrifugalizations with sterile normal salt solution.

Typhoid and cholera bacteria are killed at this stage of the procedure by heating at 56° to 60° C. for one hour. Plague bacilli are killed before the addition of the serum. By this method the endotoxins are neutralized.

Immunity in plague develops forty-eight hours after injection and lasts one and one-half months.

In typhoid and cholera injections immunity develops in twenty-four hours (with non-sensitized vaccine, it does not appear for forty-eight hours) and lasts over five months.

After injections with sensitized dysentery vaccine, immunity develops in four days and lasts over four months.

Autogenous and Stock Vaccines.—Wherever possible it is well to make the injections with cultures derived from the patient (autogenous culture). The usual practice is to use a stock vaccine for the first inoculation and autogenous vaccines later. Sometimes it is impossible to obtain a culture from the patient. The staphylococci, gonococci, tubercle, and typhoid bacilli from different cases seem much alike, so that it is less important with these organisms to get autogenous vaccines than when streptococci, pneumococci, or colon bacilli must be used.

THE DIAGNOSTIC VALUE OF OPSONINS.—The presence of a great excess or deficiency of opsonins for a microörganism, or of marked variation in the index after massage or exercise, has been thought by some to indicate the type of infection. Extreme caution should be used in making such an application of the index determinations.

Leukocyic Extract in Infections.—Hiss[2] recommends the treatment of certain diseases with a leukocyte extract. He makes this extract as follows:

The leukocytes are obtained by double pleural inoculations with aleuronat into the animal (rabbit, dog). The amount of leukocyte-filled fluid obtained after twenty-four hours from rabbits has usually been from 30 to 60 c.c. This is immediately centrifugalized, the serum poured off, and the extracting fluid (distilled water) added in amounts about equal to the fluid poured off. The cells are then thoroughly emulsified in the distilled water, allowed to stand for a few hours at 37.5° C., and then at ice-box temperature until used. Varying amounts

[1] See Rabies, Sec. III.
[2] Jour. Med. Research, xiv, No. 3.

of the entire fluid (after shaking) are inoculated. Hiss's animal experiments were made on rabbits and guinea-pigs infected with staphylococcus, streptococcus, pneumonoccus, typhoid bacillus, or meningococci. Hiss states that animals suffering from severe septicæmias and poisonings following intravenous injection of any one of the above organisms have shown the beneficial effect of treatments with extracts of leukocytes, and have, in many instances, survived infections fatal to the control animals in thirty-six hours, even when treatment has been delayed as late as twenty-four hours. Zinsser[1] has carefully studied the nature of the substances extracted from leukocytes. He finds they contain no complement, are not destroyed by heating to 56° C., and are no more abundant in cells derived from immunized than from normal rabbits.

The Hiss leukocytic extract has now been used by a number of observers for some two years. Cases of pneumonia, erysipelas, septicæmia and some other infections have been treated. It is difficult to determine just what the value of the treatment is. No harmful results have been noticed. In a certain number of cases the temperature and symptoms have bettered, in a way which seemed clearly to indicate that the extract had done good. In other cases no results whatever were apparent. It is usually given subcutaneously in 10 c.c. doses, every four to six hours. As high as 500 c.c. have been given in some cases.

Vaccines as Immunizing Agents.—The injection of vaccines in healthy subjects for the prevention of disease have been made so extensively that no one doubts the advisability of their use. Typhoid vaccines are used extensively in the army and among persons going into special danger. The usual injections are either 100 million, 300 million, and 500 million or two injections of 500 million and 800 million. Tests of the blood of such individuals show a large development of antibodies. Cholera vaccines and vaccines against bubonic plague also have been widely used.

[1] Jour. Med. Research, Vol. xxii, No. 3.

CHAPTER XV.

THE USE OF ANIMALS FOR DIAGNOSTIC AND TEST PURPOSES.

SUITABLE animals are necessarily employed for many bacteriological purposes. 1. To obtain a growth of varieties that for any reason grow with difficulty on artificial culture media, as in the case of tubercle bacilli: hence material suspected to contain tubercle bacilli is injected into guinea-pigs, with the knowledge that, if present, although in too small numbers to be detected by microscopic or culture methods, they will develop in the animals' bodies, and thus reveal themselves. The same may be true of glanders, tetanus, and anthrax bacilli, of pneumococci, of other bacteria, and of protozoa. Certain micro-organisms cannot be grown at all on artificial media. This is true of few bacteria, of most protozoa, of most of the spirochetes, and of certain unknown infectious agents such as produce smallpox and Rocky Mountain spotted fever. 2. To cause an increase of one variety of organisms in a mixture and thus obtain a pure culture: An injection of sputum subcutaneously in rabbits may give rise to a pure pneumococcus septicæmia or a pure tuberculosis. 3. To test virulence: Animals are used to test the virulence or toxin production of organisms, where, as in the case of diphtheria, we have very virulent, attenuated, and non-virulent bacilli of, so far as we know, identical cultural characteristics. Here the injection of a susceptible animal, such as the guinea-pig, is the only way that we can differentiate between those capable of producing diseases from those that are harmless. Still another use of the animals is to differentiate between two virulent organisms, which, though entirely different in their specific disease poisons, are yet so closely allied morphologically and in culture characteristics that they cannot always be separated except by studying their action in the animal body both with and without the influence of specific serums. In this way the typhoid and colon bacilli may be separated, or the pneumococcus and streptococcus. 4. To test the antitoxic or bactericidal strength of sera: Diphtheria antitoxin is added to diphtheria toxin and injected into guinea-pigs, and streptococcus immunizing serum is mixed with living streptococci and injected into the vein of a rabbit. 5. To produce antitoxic, bactericidal, or agglutinating sera.

The Inoculation of Animals.—The inoculation of animals may be made either through natural channels or through artificial ones:

1. Cutaneous. Cultures are rubbed into the abraded skin.

2. Subcutaneous. The bacteria are injected by means of a hypodermic needle under the skin, or are introduced by a platinum loop into a pocket made by an incision.

3. Intravenous. The bacteria are injected by means of a hypodermic needle into the vein. This is usually carried out in the ear vein of the rabbit. If rabbits are placed in a holder, so that the animal remains quiet and only the head projects, it is usually easy to pass a small needle directly into one of the ear veins, especially those running along the edge of the ear. If the ear is first moistened with a 3 per cent. carbolic acid solution, and then supported between the finger inside and the thumb outside, the vein is usually clearly seen and entered with ease, if a small, sharp needle is held almost parallel with the ear surface and gently pushed into it. When no holder is present, the rabbit can be held by an assistant seizing the forelegs in one hand and the hind in another and holding the rabbit head downward, or the animal may be held between the knees of the operator, its body resting on the operator's apron.

4. Into the anterior chamber of the eye.

5. Into the body cavities. The peritoneal and less often the pleural cavities are used for bacterial injection. The hypodermic needle is usually employed, less often a glass tube drawn out to a fine point. The needle or the pointed glass tube is gently pushed through the abdominal wall, moved about to be certain that the intestines have not been perforated and the fluid injected.

6. By inhalation. This method is carried out by forcing the animal to inhale an infected spray or dust.

7. By the trachea. This method is carried out by making an incision in the trachea and then inoculating the mucous membrane or injecting substances into the trachea and bronchi.

8. Through the intestinal tract by swallowing or by the passage of a rubber tube. Morphine may be given to prevent peristalsis.

9. Into the brain substance or ventricles after trephining, or when the parietal bones are thin as in the guinea-pig and the rabbit, after making a tiny opening with the point of a small, heavy scalpel.

In these injections guinea-pigs are held, as a rule, by an assistant grasping in one hand the forelegs and in the other the hind legs.

Rabbits can be held in the same manner or, they may be placed in some holder or strung up by their hind legs, or held between the knees.

Mice, which are usually inoculated subcutaneously in the body or at the root of the tail, are best placed in a mouse holder, but can be inoculated by grasping the tail in a pair of forceps, and then, while allowing the mouse to hang head downward in a jar, a glass plate is pushed across the top until only space for its tail is left.

Monkeys and apes are used for certain infections, such as syphilis and smallpox, where only man and they are markedly susceptible.

All these methods must be carried out with the greatest care as to cleanliness, the hair being clipped and the skin partially, at least, disinfected. The operator must be careful not to infect himself or his surroundings. After the inoculations the animals should be given the best of care, unless, for special purposes, we want to study

them under unusual conditions. For food, rabbits and guinea-pigs require only carrots and hay.

When possible, all animals should be anesthetized during painful experiments.

If animals die, autopsy should be made at the earliest moment possible, for soon after death some of the species of the bacteria in the intestines are able to penetrate through the intestinal walls and infect the body tissues. If delay is unavoidable, the animals should be placed immediately in a place where the temperature is near the freezing point. In making cultures from the dead bodies the greatest care should be taken to avoid contamination. The skin should be disinfected, and any dust prevented by wetting with a 5 per cent. solution of carbolic acid. All instruments are sterilized by boiling in 3 per cent. washing soda solution for five minutes. Changes of knives, scissors, and forceps should be made as frequently as the old ones become infected. When organs are examined the portion of the surface through which an incision is to be made must be sterilized, if there is danger that the surrounding cavity is infected, by searing with the flat blade of an iron spatula which has been heated to a dull red heat. Tissues if removed should be immediately placed under cover so as not to become infected. Sterile deep Petri plates are useful for this purpose.

When it is necessary to transport tissues from a distance they should be wrapped in bichloride cloths and sent to the point of destination as soon as possible. In warm weather they may be kept cool by surrounding the vessels which contain them with ice.

Animals rarely show the same gross lesions as man when both suffer from the same infection. The cell changes, however, are similar, and, also, so far as we can test them, the curative or immunizing effects of protective serums.

Leukocytes for Testing Phagocytosis.—Inoculate into the pleural cavity of a rabbit 5 c.c. of a thick suspension of aleuronat powder in a boiled starch solution. The solution should be thick enough to hold the aleuronat in suspension. A 20 to 25 per cent. solution of peptone gives good results. The fluid is withdrawn eighteen to twenty-four hours after the injection.

For purposes of obtaining the opsonic index the whole blood is taken. For description of the method see chapter on opsonins.

Leukocytes from the horse can be readily obtained by mixing the blood with 1 per cent. of sodium citrate and allowing the mixture to stand. The red cells rapidly sink and leave the leukocytes in the supernatant fluid.

CHAPTER XVI.

THE PROCURING AND HANDLING OF MATERIAL FOR BACTERIOLOGIC EXAMINATION FROM THOSE SUFFERING FROM DISEASE.

A LONG experience has taught us that physicians very frequently take a great amount of trouble, and yet, on account of not carrying out certain simple but necessary precautions, make worthless cultures or send material almost useless for bacteriologic study.

In making cultures from diseased tissues various procedures may be carried out, according to the facilities which the physician has and the kind of information that he desires to obtain. From the dead body culture material should be removed at the first moment possible after death. Every hour's delay makes the results less reliable. From both dead and living tissues, the less the alteration that occurs in any substance between its removal from the body and its examination and inoculation upon or in culture media or animals, the more exact will be the information obtained. If the material is allowed to dry many bacteria will be destroyed in the process, and certain forms which were present will be obliterated or, at least, entirely altered in the proportion which they bear to others. If possible, therefore, smears should be made and culture media should be inoculated directly from the patient or dead body. For the latter purpose a bacteriologist should take the most suitable of the culture media to the bedside or autopsy table. Such a list of media, if fairly complete, would comprise nutrient bouillon alone and mixed with one-third its quantity of ascitic fluid, slanted nutrient agar, slanted agar streaked with rabbit or human blood, firmly solidified slanted blood serum and slanted ascitic glucose agar. Additional media will be necessary for special purposes, such as the isolation of typhoid or tetanus bacilli. If only one variety of media is to be used the solidified blood serum is most useful for parasitic bacteria, and this can be easily carried by the physician and inoculated by him, even if he is not very familiar with bacteriologic technique. In the first place some of the infected material should always be smeared on a couple of clean slides or cover-glasses and allowed to dry. These can be stained and examined later, and may give much valuable information.

The material must be obtained in different ways, according to the nature of the infection.

For the detection of the bacteria causing septicæmia we are met with the difficulty that there are apt to be very few organisms present in the blood until shortly before death. It will, therefore, be almost useless to take only a drop of blood for cultures, as even when present

there may not be more than eight or ten organisms in a cubic centimetre. If cultures are to be made at all, it is, therefore, best to make them correctly by taking from 5 to 20 c.c. of blood by means of a sterile hypodermic needle or a suitable glass tube armed with a hypodermic needle from the vein of the arm, after proper cleansing of the skin and a tiny incision. To each of five different tubes containing bouillon we add 1 c.c. of blood, and to a flask containing 100 c.c. we add 5 c.c. We have made by this mixture of blood and bouillon a most suitable medium for the growth of all bacteria which produce septicæmia, and, at the same time, have added a sufficient quantity of blood to insure us the best possible chance of having added some of the bacteria producing the disease. We also add to each of several tubes of melted nutrient agar, at 40° C., 1 c.c. of blood and pour the mixture into Petri plates, so as to indicate roughly the number of organisms present if they happen to be in abundance. When blood must be carried to a distance, clotting should be prevented by having in the test-tube sufficient 10 per cent. solution of sodium citrate, bile, or ammonium oxalate to prevent clotting.

From wounds, abscesses, cellulitis, etc., the substance for bacteriologic examination can, as a rule, best be obtained by means of a syringe, or when the lesion is opened, by small rods armed with a little absorbent cotton. A number of these swabs can be sterilized in a test-tube and so carried. The swab is inserted in the wound, then streaked gently over the oblique surface of the nutrient agar in one tube, over the blood serum in another, and then inserted in the bouillon. Finally, either at the bedside or in the laboratory, material is thinly streaked over the surface of nutrient agar contained in several Petri dishes. We inoculate several varieties of media, with the hope that one at least will prove a suitable soil for the growth of the organisms present. From surface infections of mucous membranes, as in the nose, throat, vagina, etc., the swab, again, is probably the most useful instrument for obtaining the material for examination. The greatest care, of course, must be used in all cases to remove the material for study without contaminating it in any way by other material which does not belong to it. Thus, for instance, if we wish to obtain material from an abscess of the liver, where the organ lies in a peritoneal cavity infected with bacteria, one must first absolutely sterilize the surface of the liver by pressing on it the blade of a hot iron spatula before cutting into the abscess, so that we may not attribute the infection which caused the abscess to the germs which we obtained from the infected surface of the liver. From such an organ as the uterus it is only with the greatest care that we can avoid outside contamination, and only an expert bacteriologist familiar with such material will be able to eliminate the vaginal from the uterine bacteria.

A statement of the conditions under which materials are obtained should always accompany them when sent to the laboratory for examination, even if the examination is to be made by the one who made the cultures. These facts should be noted, or otherwise at some

future date they may be forgotten and misleading information sent out. The work of obtaining material for examination without contamination is at times one of extreme difficulty. It simply must be remembered that if contamination does take place our results may become entirely vitiated, and if the difficulties are so great that we cannot avoid it, it may simply mean that under such conditions no suitable examination can be made. Where the substance to be studied cannot be immediately subjected to cultures or animal inoculations, it should be transferred in a sterile bottle as soon as possible to a location where the cultures can be made. If for any reason delay must take place, the material should at least be put in a refrigerator, where cold will both prevent any further growth of some varieties of bacteria and lessen the danger of the death of others.

In obtaining samples of fluid, such as urine, feces, etc., the bottles in which they are placed should always be sterile, and, of course, no antiseptic should be added. It is necessary clearly to explain this to the nurse, for she has probably been instructed to add disinfectants to all discharges. Disinfected material is, of course, entirely useless for complete bacteriologic investigations. It cannot be too much emphasized that materials which are not immediately used should be sent to the laboratory as quickly as possible, for in such substances as fæces, where enormous numbers of various kinds of bacteria are present, those which we seek most, such as the typhoid bacilli, frequently succumb to the deleterious products of the other bacteria present. Even when abundantly present, living typhoid bacilli may entirely disappear from the fæces in the course of twelve hours, while at other times they may remain for weeks. These differences depend on the associated organisms present, the chemical constitution of the fæces or urine, and the conditions under which the material is obtained. Water and milk rapidly change in their bacterial content if not kept under 40° F.

For obtaining fluid for agglutination and other purposes, blister fluid is valuable. A blister can be raised quickly by placing a piece of blotting-paper moistened with a little strong ammonia on the skin and covering with a watch-glass, or one may be more slowly formed by a cantharides plaster.

Routine Technique Carried Out at Laboratory when Thorough Examination Required.—As has just been indicated, the bacteriological examination proceeds somewhat differently according to the information needed. When, as is the case with most clinical material, definite knowledge in regard to the presence or absence of a particular bacterium is desired, the special methods, which have been already partly given and which are later fully described under each micro-organism, are used; but when, as is generally the case with autopsy material and sometimes with clinical, a complete examination is needed, the procedure may be as follows:

1. At the autopsy table the routine cultures and smears are made as described above.

2. Material from the different parts is secured under aseptic precautions in sterile receptacles and taken to the bacteriological laboratory. The receptacles should be surrounded by ice if the laboratory is at a distance.

3. A smear from each part is stained and examined in order to determine in some measure the kind and number of bacteria present, so we may more wisely select suitable culture media, if other than those already used be needed, and may make the right culture dilutions if these be necessary.

Gram's stain (see p. 33) gives more information, especially in regard to the first point, than any other one stain, so when possible this stain should be used. Other stains, however, may help, if for any reason Gram's is not at hand; and smears made from blood or from suspected syphilitic material should be stained by Giemsa's method (see Sec. III) or an equivalent (see under malarial organisms, treponema pallidum, etc.).

A Gram-stained smear may show all Gram-negative or all Gram-positive bacteria or a mixture of the two.

The following points must be remembered in using this stain and in interpreting the results:

(*a*) The smears should be thin and evenly spread.

(*b*) The staining solutions should be fresh (aniline water, gentian violet, lasts about 3 weeks.)

(*c*) Controls of fresh cultures (about 24 hrs. old) of a Gram-negative and a Gram-positive bacterium should be used on the same slide with the smear to be examined.

(*d*) If there is much albumin in the suspected material less heat should be used in fixing.

(*e*) If the urine is very acid the results may not be good.

(*f*) Mix urinary sediment with egg albumen, better to fix it, and wash out urinary salts with tap water and stain.

(*g*) Too much dependence should not be placed upon the finding of Gram-negative bacteria in tissues, because bacteria which in pure young cultures may be positive to Gram, may, as they grow older both in tissues and in cultures, show forms intermediate between negative forms, as well as a varying number of the latter.

If the smears show only Gram-negative organisms, the material probably contains one or more of the following:

Gram-Negative Bacilli.

- B. coli group. — Most frequently from intestinal tract.
- B. typhosus group. — Most frequently from intestinal tract.
- B. dysenteriæ group. — Most frequently from intestinal tract.
- B. proteus. — Most frequently from intestinal tract.
- B. mucosus capsulatus. — Most frequently from intestinal tract.
- B. pyocyaneus. — Most frequently from chest contents.
- B. influenzæ group. — Most frequently from chest contents.
- B. fusiformis. — Most frequently from chest contents.

  → Most frequently found, and some indication of their presence in history.

- B. mallei.
- B. edematis (malignant œdema).
- B. of symptomatic anthrax.
- B. pestis.
- B. of Morax-Axenfeld.

  → Less frequently found, and generally a marked indication of their presence in history.

Gram-Negative Cocci.	Micrococcus intracellularis. Micrococcus catarrhalis. Micrococcus gonorrhœæ. Micrococcus melitensis.	Generally marked indication of their presence in history.
Gram-Negative Spirilla.	S. choleræ and allied forms.	Marked indication of presence of first form in history.
	Mouth spirals.	Unimportant, unless indication of syphilis in history when Tr. pallidum should be looked for.

If only Gram-positive organisms are demonstrated, the material may contain one or more of the following:

Gram-Positive Bacilli.	B. diphtheriæ group. B. tetani (not often demonstrated in smears from lesion). B. tuberculosis. B. anthracis. B. leprae. B. welchii and some other intestinal anaërobes.	Generally marked indication of their presence in history.
Gram-Positive Cocci.	Staphylococcus group. Streptococcus group (including pneumococcus and its variety, pneumococcus mucosus). Micrococcus tetragenus.	Some indication of their presence in history.
Gram-Positive Spirilla.	None.	

From the different parts of the body the following more important organisms are found in order of their probable frequency.

Serous Fluids.	Meningeal (Cerebrospinal).	Micrococcus intracellularis. Streptococcus (including pneumococcus group). B. influenzæ.	Fluid generally cloudy with many leukocytes.
		B. tuberculosis	Fluid generally clear.
	Pericardial and pleural.	Streptococcus (including pneumococcus group). B. mucosus capsularis. B. influenzæ.	Fluid may be cloudy.
		B. tuberculosis.	Fluid generally clear.
	Peritoneal.	B. coli group. Streptococcus group. B. tuberculosis.	
Lungs.	Streptococcus (including pneumococcus group). B. mucosus capsularis. B. influenzæ. B. tuberculosis.		

Nose and Throat.	B. diphtheriæ group. B. influenzæ group. Streptococcus group. B. mucosus group. B. tuberculosis.
Fæces.	B. coli group (including B. fæcalis alcaligenes and B. acidi lactici). B. typhosus group. B. dysenteriæ group. Gram-positive anaërobes. Many forms whose importance has not been worked out.
Urine.	B. coli group. Streptococcus (kidney). M. gonorrhœæ. B. typhosus. B. tuberculosis.
Pelvic Organs.	M. gonorrhœæ. Streptococcus. B. tuberculosis. Many other forms probably unimportant.

The following media should be used for the reasons given below:

Nutrient broth, for motility, morphology, and arrangement (chains, groups, etc.).

Potato for color and abundance of growth.

Peptone broth for indol.

Fermentation tube for anaërobes, acidity and gas.

Nutrient Agar and Gelatin.	(*a*) Poured plates for isolated colonies (dilutions according to the number of organisms seen in smears). (Blood agar if pneumococcus or streptococcus indicated.) (*b*) Streaked plates for surface colonies. (Blood agar if influenza bacilli are indicated.)

Special media according to the kinds of organisms demonstrated in smears or indicated in histories. Such special media are described under the individual organisms.

PART II.

BACTERIA PATHOGENIC TO MAN INDIVIDUALLY CONSIDERED.

CHAPTER XVII.

THE BACILLUS AND THE BACTERIOLOGY OF DIPHTHERIA.

THE lesions of diphtheria are caused by toxæmia. The concentrated poison at the seat of the exudate causes intense local inflammation, while the absorbed poison diffused throughout the body causes widespread cellular injury, giving rise to constitutional disturbance and definite injury of the cells of the muscle, nerve, and other tissues.

Historical Notes.—This specific contagious disease can be traced back under various names to almost the Homeric period of Grecian history. From time to time during the following centuries we hear of epidemics both in Italy and in other portions of the civilized world which indicate that the disease never absolutely ceased.

In 1765 Home, a Scotchman, tried to show that "croup" and pharyngeal diphtheria were different diseases.

In 1771 Bard, an American, supported the opposite theory from Home, considering the process the same wherever located.

In 1821 Bretonneau published his first essay on diphtheria in Paris and gave to the disease its present name. His observations were so extensive and so correct that little advance in knowledge took place until the causal relations of the diphtheria bacilli and their associated micoörganisms to the disease began to be recognized.

Evidence of Causal Relationship.—As early as 1840 observers began to notice microörganisms in the pseudomembranes. Gradually the observations became more exact. The most importance was attributed to micrococci. In the year 1883, however, bacilli which were very peculiar and striking in appearance were shown by Klebs to be of constant occurrence in the pseudomembranes from the throats of those dying of true epidemic diphtheria. He described the peculiar staining of the organisms. One year later, Loeffler separated these bacilli from the other bacteria and grew them in pure culture. When he inoculated the bacilli upon the abraded mucous membrane of susceptible animals more or less characteristic pseudomembranes were

produced, and frequently death or paralysis followed with characteristic lesions. These animal experiments have been fortified by a number of accidental human infections with bacilli in laboratories with subsequent development of diphtheria.

The Diphtheria Bacillus.—This bacillus is one of the most interesting of bacteria. Grown in the animal body or in suitable culture fluid, it produces a powerful toxin. Its morphology and staining are peculiar. Outside of the body it grows best on serum media.

Morphology.—When cover-glass preparations made from the exudate or from the cultures grown on blood serum are examined, the diphtheria bacilli are found to possess the following morphological characteristics: The diameter of the bacilli varies from 0.3 to 0.8μ and the length from 1 to 6μ. They occur singly and in pairs (see Figs. 77 to 84) and very infrequently in chains of three or four. The rods are straight or slightly curved, and usually are not uniformly cylindrical throughout their entire length, but are swollen at the end, or pointed at the ends and swollen in the middle portion. The average length of the bacilli in pure cultures from different sources frequently varies greatly, and even from the same culture individual bacilli differ much in their size and shape. This is especially true when the bacilli are grown in association with other bacteria. The two bacilli of a pair may lie with their long diameter in the same axis or at an obtuse or an acute angle. The bacilli possess no spores, but have in them highly refractive bodies, some of which are the starting point for new bacilli (see p. 16). There are no flagella. For mode of division, see p. 16.

Staining.—The Klebs-Loeffler bacilli *stain* readily with ordinary aniline dyes, and retain fairly well their color after staining by Gram's method. With Loeffler's alkaline solution of methylene blue, and to a less extent with Roux's and dilute Ziehl's solutions, the bacilli from blood serum cultures especially, and from other media less constantly, stain in an irregular and extremely characteristic way. (See Fig. 77.) The bacilli do not stain uniformly. In many cultures round or oval bodies, situated at the ends or in the central portions, stain much more intensely than the rest of the bacillus, usually showing metachromatism (the so-called metachromatic granules. See p. 14). Sometimes these highly stained bodies are thicker than the rest of the bacillus; again, they are thinner and surrounded by a more slightly stained portion. Other bacilli have barred staining. The bacilli stain in this peculiar manner at a certain period of their growth, so that only a portion of the organisms taken from a culture at any one time will show the characteristic staining. The young cultures have the most regular forms, an eighteen-hour growth showing more clubbed forms than at twelve hours. After twenty-four hours the bacilli do not stain quite as well. In still older cultures it is often difficult to stain the bacilli, and the staining, when it does occur, is frequently not at all characteristic. The same round or oval bodies which take the methylene blue more intensely than the remainder of the bacillus are brought out still more distinctly by the Neisser stain.

The Neisser stain is carried out by placing the cover-slip smear of diphtheria or other bacilli in solution No. 1 for from two to three seconds, and then, after washing, in No. 2 for from three to five seconds. The bacilli will then appear either entirely brown or will show at one or both ends a dark blue, round body. With characteristic diphtheria bacilli, taken from a twelve

FIG 77

FIG. 78

FIG. 77.—One of the very characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain. ×1100 diameters. Stain, methylene blue.
FIG. 78.—Extremely long form of diphtheria bacillus. This culture has grown on artificial media for fifteen years and produces great amounts of toxin. ×1100 diameters.

FIG. 79

FIG. 80

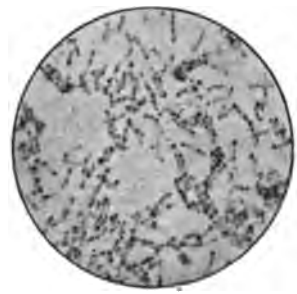


FIG. 79.—Diphtheria bacilli characteristic in shapes, but showing even staining. ×1000 diameters. Stain, methylene blue.
FIG. 80.—Non-virulent diphtheria bacilli, showing stain with Neisser's solutions. This appearance was formerly supposed to be characteristic of virulent bacilli. Bodies of bacilli in smear, yellowish-brown; points, dark blue.

to eighteen hours' growth on serum, nearly all will show the blue bodies (Fig. 80), while with the pseudo type (Fig. 86, page 206), to be described hereafter, few will be seen.

The solutions are as follows:

No. 1.

Alcohol (96 per cent.)	20 parts.
Methylene blue (Grübler)	1 part.
Distilled water	950 parts.
Acetic acid (glacial)	50 parts.

No. 2.

Bismarck brown	1 part.
Boiling distilled water	500 parts.

The Neisser stain has been advocated in order to separate the virulent from the non-virulent bacilli, without the delay of inoculating

animals; but in our hands, with a very large experience, neither the Neisser stain nor other stains, such as the modifications of the Roux stain, have given much more information as to the virulence of the bacilli than the usual methylene-blue solution of Loeffler. A few strains of virulent bacilli fail to show a marked characteristic stain, and quite a few pseudodiphtheria bacilli show the dark bodies. There are also in many throats bacilli which seem to have all the staining

FIG. 81

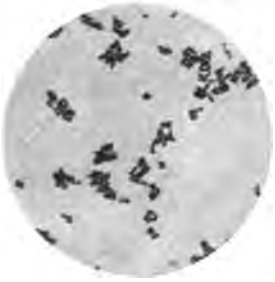


FIG. 82

FIG. 81.—B. diphtheria agar culture. Bacilli small and uniform in shape. ×1000 diameters.
FIG. 82.—B. diphtheriæ. Forty-eight hours' agar culture. Thick, Indian-clubbed rods and moderate number of segments. One year on artificial culture media. ×1410 diameters.

FIG. 83

FIG. 84

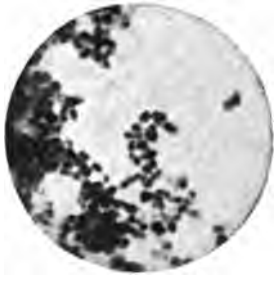


FIG. 83.—B. diphtheriæ. Forty-eight hours' agar culture. Many segments; long, Indian-clubbed ends. One year on artificial media. ×1410 diameters.
FIG. 84.—B. diphtheriæ. Twenty-four hours' agar culture. Coccus forms. Segmented granular forms on Loeffler's serum. Only variety found; in cases of diphtheria at Children's Home. ×1410 diameters.

and cultural characteristics of the virulent bacilli, and yet have no relation to the disease diphtheria. They are therefore non-virulent in the sense that they produce no diphtheria toxin. As will be stated more fully later, nothing but animal inoculations with the suspected bacilli together with control injections of diphtheria antitoxin will separate harmless bacilli from those capable of producing diphtheria.

The Morphology of the Diphtheria Bacillus on Serum-free Media.—This varies considerably with different culture media employed. On glycerin agar or simple nutrient agar there are two distinct types. One grows as smaller and, as a rule, more regular forms than when grown on serum culture media (Fig. 81). The other type shows many thick, Indian-clubbed forms with a moderate number of segments (Figs. 82–84). Short, spindle,

lancet, or club-shaped forms, staining uniformly, are all observed. The bacilli which have developed in the pseudomembranes or exudate in cases of diphtheria resemble in shape young bacilli grown on blood serum, but stain more evenly.

Biology.—The Klebs-Loeffler bacillus is non-motile and non-liquefying. It is *aërobic*. It grows most readily in the presence of oxygen, but also without it. It does not form spores. It begins to develop, but grows slowly at a temperature of 20° C., or even less. It attains its maximum development at 37° C. In old cultures in fluid media, Williams has observed fusion of one bacillus with another. The fused forms live the longest (see p. 18).

Growth on Culture Media.—Blood Serum.—Blood serum, especially coagulated in the form of Loeffler's mixture, is the most favorable medium for the growth of the diphtheria bacillus, and is used particularly for diagnostic purposes in examining cultures from the throats of persons suspected of having diphtheria. For its preparation, see p. 227. If we examine the growth of diphtheria bacillus in pure culture on blood serum we shall find at the end of from eight to twelve hours small colonies of bacilli, which appear as pearl-gray, whitish-gray or, more rarely, yellowish-gray, slightly raised points. The colonies when separated from each other may increase in forty-eight hours so that the diameter may be one-eighth of an inch. The borders are usually somewhat uneven. The colonies lying together become confluent and fuse into one mass when the serum is moist. During the first twelve hours the colonies of the diphtheria bacilli are about equal in size to those of the other pathogenic bacteria which are often present in the throat; but after this time the diphtheria colonies become larger than those of the streptococci and smaller than those of the staphylococci. The diphtheria bacilli in their growth never liquefy blood serum.

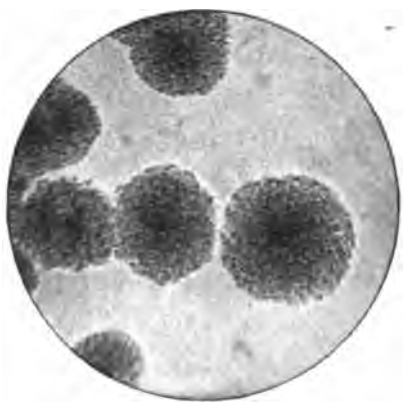
Growth on Agar.—On 1 per cent. slightly alkaline, nutrient or glycerin-agar the growth of the diphtheria bacillus is less certain and luxuriant than upon blood serum; but the appearance of the colonies when examined under a low-power lens, though very variable, is often far more characteristic. (See Fig. 53, page 75, and Fig. 85.) For this reason nutrient agar in Petri dishes is used to obtain diphtheria bacilli in pure culture. The diphtheria bacillus obtained from cultures which have developed for some time on culture media grows well, or fairly well, on suitable nutrient agar, but when fresh from pseudomembranes one prevalent type of bacilli grows on these media with great difficulty, and the colonies develop so slowly as to be frequently covered up by the more luxuriant growth of other bacteria when present, or they may fail to develop at all.

If the colonies develop deep in the substance of the agar they are usually round or oval, and, as a rule, present no extensions; but if near the surface, commonly from one, but sometimes from both sides, they spread out an apron-like extension, which exceeds in surface area the rest of the colony. When the colonies develop entirely on the

surface they are more or less coarsely granular, and usually have a dark centre and vary markedly in their thickness. The colonies from some are almost translucent; from others are thick and almost as luxuriant as the staphylococcus. The edges are sometimes jagged, and frequently shade off into a delicate lace-like fringe; at other times the margins are more even and the colonies are nearly circular.

The growth of the diphtheria bacillus upon agar presents certain peculiarities which are of practical importance. If a large number of the bacilli from a recent culture are implanted upon a properly prepared agar plate a certain and fairly vigorous growth will always take place. If, however, the agar is inoculated with an exudate from the throat, which contains but a few bacilli, no growth whatever may occur, while the tubes of coagulated blood serum inoculated with the same exudate contain the bacilli abundantly. Because of the uncertainty, therefore, of obtaining a growth by the inoculation of agar with bacilli unaccustomed to this medium, agar is not a reliable medium for use in primary cultures for diagnostic purposes. A mixture composed of two parts of a 1.5 per cent. nutrient agar and one part of sterile ascitic fluid makes a medium upon which the bacillus grows much more luxuriantly, but not so characteristically.

FIG. 85



Colonies of diphtheria bacilli. ×200 diameters.

Isolation of the Diphtheria Bacillus from Plate Cultures.—Nutrient plain or glycerin-agar should be freshly melted and poured in the Petri dish for this purpose. After it has hardened, the medium in a number of plates is streaked across with bacteria from colonies on the serum culture, which appear in size and color like the diphtheria bacilli. Other plates are made from a general mixture of all bacteria, selected, as a rule, from the drier portion of the serum. Still others are inoculated from the pellicles of ascitic broth cultures. The plates are left in the incubator for about sixteen hours at 37° C. In the examination of the plates one should first seek for typical colonies, then if these are not found, for any that look most nearly like the characteristic picture. Diphtheria colonies are very apt to be found at the edges of the streaks of bacterial growth. The pickings from the colonies are inoculated upon Loeffler's blood serum, or into ascitic bouillon.

Growth in Bouillon.—The diphtheria bacilli from about one-half the cultures grow readily in broth slightly alkaline to litmus; the other cultures grow feebly. The characteristic growth in neutral bouillon is one showing fine grains. These deposit along the sides and bottom of the tube, leaving the broth nearly clear. A few cultures in neutral bouillon and many in alka-

line bouillon produce for twenty-four or forty-eight hours a more or less diffuse cloudiness, and frequently a film forms over the surface of the broth. On shaking the tube this film breaks up and slowly sinks to the bottom. This film is apt to develop during the growth of cultures which have long been cultivated in bouillon, and, indeed, after a time the entire development may appear on the surface in the form of a friable pellicle. The diphtheria bacillus in its growth causes a fermentation of meat-sugars and glucose, and thus if these are present changes the reaction of the bouillon, rendering it distinctly less alkaline within forty-eight hours, and then, after a variable time, when all the fermentable sugars have been decomposed, more alkaline again through the progressing fermentation of other substances. Among the products formed by its growth is the diphtheria toxin.

Growth in Ascitic or Serum Bouillon.—All varieties of diphtheria bacilli grow well in this medium, even when first removed from the throat. They almost always form a slight pellicle at the end of twenty-four to forty-eight hours. This culture medium is, as pointed out by Williams, of the greatest value in attempts to get pure cultures of the diphtheria bacillus from solidified serum cultures containing few bacilli among many other bacteria. Plate cultures are made from the pellicle. The fluid is prepared by adding to the nutrient bouillon 25 per cent. ascitic fluid or blood serum.

Growth on Gelatin.—The growth on this medium is much slower, more scanty, and less characteristic than that on the other media mentioned. This is partly on account of the lower temperature at which it must be used.

Growth in Milk.—The diphtheria bacillus grows readily in milk, beginning to develop at a comparatively low temperature (20° C.). Thus, market milk having become inoculated with the bacillus from cases of diphtheria may, under certain conditions, be the means of conveying infection to previously healthy persons. The milk remains unchanged in appearance as lactose is not fermented by the diphtheria bacillus.

Pathogenesis. In Lower Animals.—The diphtheria bacillus through its toxins is, when injected into their bodies, pathogenic for guinea-pigs, rabbits, chickens, pigeons, small birds, and cats; also in a lesser degree for dogs, goats, cattle, and horses, but hardly at all for rats and mice. In spite of its pathogenic qualities for these animals true diphtheria occurs in them with extreme rarity. As a rule, supposed diphtheritic inflammations in them are due to other bacteria which cannot produce the disease in man. The cat is the only animal that we have known to contract true diphtheria from contact with the disease. Cobbett reports a case in a colt. At the autopsy of animals dying from the poisons produced by the bacilli, the characteristic lesions described by Loeffler are found. At the seat of inoculation there is a grayish focus surrounded by an area of congestion; the subcutaneous tissues for some distance around are œdematous; the adjacent lymph nodes are swollen; and the serous cavities, especially the pleura and the pericardium, frequently contain an excess of fluid usually clear, but at times turbid; the lungs are generally congested, the suprarenals are markedly congested. In the organs are found

numerous smaller and larger masses of necrotic cells, which are permeated by leukocytes. The heart and certain voluntary muscular fibres and nervous tissues usually show degenerative changes. Occasionally there is fatty degeneration of the liver and kidneys. The number of leukocytes in the blood is increased. From the area surrounding the point of inoculation virulent bacilli may be obtained, but in the internal organs they are only occasionally found, unless an enormous number of bacilli have been injected. Paralysis, commencing usually in the posterior extremities and then gradually extending to the whole body and causing death by paralysis of the heart or respiration, is also produced in many cases in which the inoculated animals do not succumb to a too rapid intoxication. In a number of animals we have seen recovery take place three to six weeks after the onset of the paralysis. The occurrene of these paralyses, following the introduction of the diphtheria bacilli, completes the resemblance of the experimental disease to the natural malady in man.

Tissue Changes in Natural (Human) Infection.—The characteristic lesions are a pseudomembranous inflammation on some of the mucous membranes or occasionally on the surface of wounds and the general hyperplasias and parenchymatous inflammations produced by the absorbed toxic substances. Pneumonia is apt to occur as a complication of laryngeal diphtheria. The membrane may be simply a thin pellicle, which is easily removed without causing bleeding or it may be thick and firmly attached and leaving when removed a ragged bleeding surface. The tissue beneath the pseudomembrane is always intensely injected and often hemorrhagic. The cells show marked degenerative changes.

Causes of Death.—These are chiefly toxæmia, septicæmia, laryngeal obstruction and broncho-pneumonia.

Diphtheria Toxin.—This poison was assumed by Loeffler (1884) to be produced by the bacilli, but it was first partially isolated by Roux and Yersin, who obtained it by filtration through porous porcelain from cultures of the living bacilli. It has not yet been successfully analyzed, so that its chemical composition is unknown, but it has many of the properties of proteid substances, and can well be designated by the term active proteid. It resembles in many ways the ferments. After injection into the body there is a latent period before its poisonous action appears. The poison produced is probably composed of a mixture of several nearly related toxins. Diphtheria toxin is totally destroyed by boiling for five minutes, and loses some 95 per cent. of its strength when exposed to 75° C. for the same time; 73° C. destroys only about 85 per cent., and 60° very little. Lower temperatures only alter it very gradually. Kept cool and from light and air, it deteriorates very slowly. Freezing injures it.

The Production of Toxin in Culture Media.—The artificial production of toxin from cultures of the diphtheria bacillus has been found to depend upon definite conditions, which are of practical importance in obtaining toxin for the inoculation of horses, and also of theoretic interest in explaining why cases

of apparently equal local severity have such different degrees of toxic absorption. The researches of Roux and Yersin laid the foundation of our knowledge. Their investigations have been continued by Theobald Smith, Spronck, ourselves, and others. After an extensive series of investigations we (Park and Williams) came to the following conclusions: Toxin is produced by fully virulent diphtheria bacilli at all times during their life when the conditions are favorable. Under less favorable conditions some bacilli are able to produce toxin while others are not. Diphtheria bacilli may find conditions suitable for luxuriant growth, but unsuitable for the production of toxin. The requisite conditions for good development of toxin, as judged by the behavior of a number of cultures, are a temperature from about 32° to 37° C., a suitable culture medium, such as a 2 per cent. peptone nutrient bouillon made from veal, of an alkalinity which should be about 9 c.c. of normal soda solution per litre above the neutral point to litmus, and prepared from a suitable peptone (Witte) and meat. The culture fluid should be in comparatively thin layers and in large-necked Erlenmeyer flasks, so as to allow of a free access of air. The greatest accumulation of toxin in bouillon is after a duration of growth of the culture of from five to ten days, according to the peculiarities of the culture employed. At a too early period toxin has not sufficiently accumulated; at a too late period it has begun to degenerate. In our experience the amount of muscle-sugar present in the meat makes no appreciable difference in the toxin produced when a vigorously growing bacillus is used, so long as the bouillon has been made sufficiently alkaline to prevent the acid produced by the fermentation of the sugar from producing in the bouillon an acidity sufficient to inhibit the growth of the bacilli. With the meat as we obtain it in New York, we get better results with unfermented meat than with fermented. In Boston, with the same bacillus, Smith gets more toxin from the bouillon in which the sugar has been fermented by the colon bacillus. Instead of colon bacilli, yeast may be added to the soaking meat, which is allowed to stand at about 25° C. We have obtained especially good results with veal broth made from calves two to four weeks old (bob veal). When strong toxin is desirable the muscle is separated from all fat, tendon and fibrous tissue before being chopped.

Under the best conditions we can devise, toxin begins to be produced by bacilli from some cultures when freshly sown in bouillon some time during the first twenty-four hours; from other cultures, for reasons not well understood, not for from two to four days. In neutral bouillon the culture fluid frequently becomes slightly acid and toxin production may be delayed for from one to three weeks. The greatest accumulation of toxin is on the fourth day, on the average, after the rapid production of toxin has commenced. After that time the number of living bacilli rapidly diminishes in the culture, and the conditions for those remaining alive are not suitable for the rapid production of toxin. As the toxin is not stable at 35° C., the deterioration taking place in the toxin already produced is greater than the amount of new toxin still forming.

Bacilli, when repeatedly transplanted from bouillon to bouillon, gradually come to grow on the surface only. This characteristic keeps the bacilli in contact with the oxygen and seems to aid in the development of toxin.

Comparative Virulence of Different Cultures.—The virulence of diphtheria bacilli from different sources, as measured by their toxin production, varies considerably. Thus, as an extreme instance, 0.002 c.c. of a forty-hour bouillon culture of our most virulent bacillus will kill a guinea-pig, which it would require 0.1 c.c. of the culture of our least virulent bacillus to kill. This difference frequently depends on the unequal growth of the bacilli, one culture having fifty times as many bacilli as the other. When the different strains are

grown on ascitic broth, upon which their growth is usually good, the majority of cultures are nearly equal in virulence, but some still show marked differences. Moreover, the diphtheria bacilli differ in the tenacity with which they retain their virulence when grown outside the body. The bacillus that we have used to produce toxin in the laboratory of the Board of Health has retained its virulence unaltered for fifteen years in bouillon cultures. Other bacilli have apparently lessened their capacity for toxin production after being kept six months. The passage of diphtheria bacilli through the bodies of susceptible animals does not increase their toxin production to any considerable extent.

Comparative Virulence of Bacilli and Severity of Case.—From the severity of an isolated case the virulence of the bacilli cannot be determined. The most virulent bacillus we have ever found was obtained from a mild case of diphtheria simulating tonsillitis. Another case, however, infected by the bacillus proved to be very severe. In localized epidemics the average severity of the cases probably indicates roughly the virulence of the bacillus causing the infection, as here the individual susceptibility of the different persons infected would, in all likelihood, when taken together, be similar to that of other groups; but even in this instance special conditions of climate, food, or race may influence certain localities. Moreover, the bacteria associated with the diphtheria bacilli, and which are liable to be transmitted with them, may influence the severity of and the complications arising in the cases. It must be remembered that bacilli of like toxic power may differ in their liability to infect the mucous membrane. Virulence has thus two distinct meanings when used in connection with diphtheria bacilli.

Virulent Bacilli in Healthy Throats.—Fully virulent bacilli have frequently been found in healthy throats of persons who have been brought in direct contact with diphtheria patients or infected clothing without contracting the disease. It is, therefore, apparent that infection in diphtheria, as in other infectious diseases, requires not only the presence of virulent bacilli, but also a susceptibility to the disease, which may be local or general. Among the predisposing influences which contribute to the production of diphtheritic infection may be mentioned the breathing of foul air and living in overcrowded and ill-ventilated rooms, impure food, certain diseases, more particularly catarrhal inflammations of the mucous membranes, and depressing conditions generally. Under these conditions an infected mucous membrane may become susceptible to disease. In connection with Beebe (1894) we made an examination of the throats of 330 healthy persons who had not come in contact, so far as known, with diphtheria, and we found virulent bacilli in 8, only 2 of whom later developed the disease. In 24 of the 330 healthy throats nonvirulent bacilli or attenuated forms of the diphtheria bacillus were found. Very similar observations have since been made in Boston and by others in many widely separated countries. In 1905 Von

Sholly in our laboratory examined 1000 throats of those who had not knowingly been in contact with diphtheria and found true diphtheria bacilli in 0.5 per cent. of the cases.

Persistence of Diphtheria Bacilli in the Throat.—The continued presence of virulent diphtheria bacilli in the throats of patients who have recovered from the disease has been demonstrated by all investigators. In the investigations of 1894 we found that in 304 of 605 consecutive cases the bacilli disappeared within three days after the disappearance of the pseudomembrane; in 176 cases they persisted for seven days, in 64 cases for twelve days, in 36 cases for fifteen days, in 12 cases for three weeks, in 4 cases for four weeks, and in 2 cases for nine weeks. Since then we have met with a case in which they persisted with full virulence for eight months. It is safe to say that in over 5 per cent. of the cases a few bacilli persist two weeks after the disappearance of the exudate and in over 1 per cent. four weeks.

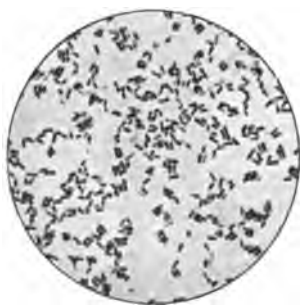
Diphtheria-like Bacilli Not Producing Diphtheria Toxin.—In the tests of the bacilli obtained from hundreds of cases of suspected diphtheria which have been carried out during the past fiifteen years in the laboratories of the Health Department of New York City, in over 95 per cent. of cases the bacilli derived from exudates or pseudomembranes and possessing the characteristics of the Loeffler bacilli have been found to be virulent, that is, producers of diphtheria toxin. But there are, however, in inflamed throats as well as in healthy throats, either alone or associated with the virulent bacilli, occasionally bacilli which, though morphologically and in their behavior on culture media identical with the Klebs-Loeffler bacillus, are yet producers, at least in artificial culture media and the usual test animals, of no diphtheria toxin. Between bacilli which produce a great deal of toxin and those which produce none we find a few minor grades of virulence. We believe, therefore, in accordance with Roux and Yersin these non-virulent bacilli should be considered as possibly attenuated varieties of the diphtheria bacillus which have lost their power to produce diphtheria toxin. This supposition is, however, not proven and it may be that the ancestors of these bacilli were never toxin producers. These observers, and others following them, have shown that the virulent bacilli can be artificially attenuated; but the reverse has not been proven that bacilli which produce no specific toxin have later been found to develop it. In our experience some cultures hold their virulence even when grown at 41° C. for a number of months, while others become partly attenuated rather quickly. We have never yet been able to change a virulent culture into an absolutely non-virulent one. Diphtheria-like bacilli are also found which resemble diphtheria bacilli very closely except in toxin production, but differ in one or more particulars. Both these and the characteristic non-virulent bacilli are found occasionally upon all the mucous membranes both when inflamed and when apparently normal. From varieties of this sort having been found in a number of cases of the condition known as xerosis con-

junctivæ, these bacilli are often called xerosis bacilli. Under this name different observers have placed bacilli identical with the diphtheria bacilli and others differing quite markedly from them.

Diphtheria-like Bacilli Pathogenic to Guinea-pigs Producing no Diphtheria Toxin.—These bacilli are obtained frequently from normal or slightly inflamed throats and may be slightly pathogenic in guinea-pigs, since they may kill, as we have found in a number of instances, in doses of 2 to 5 c.c. of broth culture subcutaneously or intraperitoneally injected. Animals are not protected by diphtheria antitoxin from the action of these bacilli. At autopsy the bacilli are usually found more or less abundantly in the blood and internal organs. The fact that large injections of antitoxic serum hastens the death of guinea-pigs injected with these bacilli has given rise to the notion that injections of antitoxin might be dangerous in persons in whose throats these bacilli were present, either as saprophytes or, possibly, as inciters of slight disease. It is not the antitoxin, but the serum, which in large doses injures the vitality of the guinea-pigs and so slightly hastens death. Any serum has the effect. These bacilli were first described by Davis[1] from our laboratory and later by Hamilton in 1904. In our judgment the possibility of their being present affords no reason to avoid giving antitoxin in suspected cases. They should in this respect be considered as the streptococci. When pathogenic in man they are usually only feebly so.

Pseudodiphtheria Bacilli.—Besides the typical bacilli which produce diphtheria toxin and those which do not, but which, so far as we can determine, are otherwise identical with the Loeffler bacillus, there are other bacilli found in positions similar to those in which diphtheria bacilli abound, which, though resembling these organisms in many particulars, yet differ from them as a class in others equally important. The variety most prevalent is rather short, plump, and more uniform in size and shape than the true Loeffler bacillus (Fig. 86). On blood-serum their colony growth is very similar to that of the diphtheria bacilli. The great majority of them in any young culture show no polar granules when stained by the Neisser method, and stain evenly throughout with the alkaline methylene-blue solution. They do not produce acid by the fermentation of glucose, as do all known virulent and many non-virulent diphtheria bacilli; therefore, there is no increase in acidity in the bouillon in which they are grown during the first twenty-four hours from the fermentation of the meat-sugar regularly present. They are found in varying abundance in different localities in New York City, in about 1 per cent. of the normal throat and nasal secretions, and seem to have now at least no connection

FIG. 86



Pseudodiphtheria bacilli. (B. hofmanni.)

[1] Medical News, April 29, 1899.

with diphtheria; whether they were originally derived from diphtheria bacillus is doubtful; they certainly seem to have no connection with it now. They have been called *pseudodiphtheria bacilli*, and more properly, *B. hofmanni*.[1] In bouillon they grow, as a rule, less luxuriantly than the diphtheria bacilli, and never produce diphtheria toxin. Some of the varieties of the pseudodiphtheria bacilli are as long as the shorter forms of the virulent bacilli. When these are found in cultures from cases of suspected diphtheria they may lead to an incorrect diagnosis. There are also some varieties which resemble the short pseudobacilli in form and staining, but which produce acid in glucose bouillon. These bacilli are found occasionally in all countries where search has been made for them. It may be added here that no facts have come to light which indicate that bacilli which do not produce diphtheria toxin in animals ever produce it in man. It must also be borne in mind, however, that such proof is necessarily very difficult to obtain.

Persistence of Varieties of the Bacillus Diphtheriæ and of Diphtheria-like Bacilli.—The fact that there are distinct differences between strains of bacilli producing specific diphtheria toxins which are as great as between these and bacilli producing no specific toxins has, we think, been fully established.

But that such varieties are true sub-species with constant characteristics, one variety not changing into another of the established forms, has not been accepted by all. On the contrary, the opinion is held by some investigators that all of the various forms of diphtheria-like bacilli are the result of more or less transitory variations of the same species, and hence that the virulent forms are the result of a rapid adaptation to environment and consequent pathogenesis of the non-virulent forms, both typical and atypical.

This question of the relationship of the specifically virulent diphtheria bacillus to non-virulent, diphtheria-like bacilli has been discussed since 1887. It is certainly theoretically possible that the non-virulent forms have been derived from virulent forms. Whether or not this is true is an interesting problem for discussion, but has little practical importance. On the other hand, the possibility of the non-toxin producing forms readily assuming power to produce toxin is of the greatest importance, and if true would cause us to change our present methods of trying to prevent the spread of diphtheria.

Until 1896 no one had brought forward evidence which tended to show that fully non-virulent forms could be made virulent. In this year Trump[2] states that he converted a non-virulent acid-producing bacillus into one capable of killing guinea-pigs with all the symptoms of true diphtheria, by successive passages through guinea-pigs plus a non-fatal dose of diphtheria toxin. Hewlett and Knight[3] state (1897)

[1] Clark. Jour. of Inf. Dis., 1910, vii., 335.
[2] Centralblatt für Bakt., etc., 1896, Band xx., p. 721.
[3] Trans. of the Brit. Inst. of Prev. Med., 1897, 1st series.

that they changed a non-acid pseudodiphtheria bacillus into a typical virulent diphtheria bacillus by culture and passage through guinea-pigs.

Richmond and Salter[1] (1898) and Salter[2] (1899) state that they have changed five pseudodiphtheria bacilli into typical diphtheria bacilli specifically virulent for guinea-pigs by passage through a number of goldfinches.

In the work of Wesbrook, Wilson, and McDaniel,[3] on *Varieties of Bacillus Diphtheriæ*, the study is based upon the morphology of the individual bacillus found in smears of throat cultures and pure cultures. They make a provisional classification based upon the morphology of the individual bacilli, into three groups, called granular, barred, and solid, two of the groups into seven types and the other into five, two of the types corresponding with those in the other groups not having been seen. In a study of the types found in the smears from a series of direct cultures derived from clinical cases of diphtheria the authors state that there is generally a sequence of types in the variations which appear throughout the course of the disease, the granular types, as a rule, predominating at the outset of the disease, and these giving place wholly or in part to the barred and solid types shortly before the disappearance of diphtheria-like organisms.

The inference drawn from this work is that the diphtheria bacillus may be rather easily, especially in the throat, converted into non-granular, solidly staining forms of the "pseudodiphtheria" type, and that the converse may occur, and that therefore all diphtheria-like bacilli must be considered a possible source of danger. The more extreme views of Hewlett and Knight are rejected by most investigators and even the conclusions of Wesbrook are considered too extreme.

Bergey[4] was not able to give virulence to non-virulent forms, neither did he find that these latter gave immunity against the former; for these reasons he considers them distinct members of a large group of bacilli at the head of which stands the diphtheria bacillus.

Cobbett[5] considers the pseudodiphtheria bacillus as perfectly innocuous to man, but that the relation between the pseudodiphtheria and the diphtheria bacillus remains undecided. He did not meet with bacilli of low virulence. He found a few non-virulent and the others all highly virulent. He thinks that the reason why the pseudo-diphtheria bacilli appear so infrequently during the acute stage is that they are overlooked then because one discovers the virulent bacilli so easily and does not trouble to look any more, and they are found more easily later because the diphtheria bacilli

[1] Guy's Hospital Reports, 1898.
[2] Trans of the Jenner Inst. of Prev. Med., 1899.
[3] Transactions of the Association of American Physicians, 1900.
[4] Pub. of the Univ. of Penn., 1898, new series, No. 4 (other references).
[5] Journal of Hygiene, 1901.

are disappearing and are hard to find; consequently a long and careful search is made, and the pseudodiphtheria bacilli are seen for the first time.

The central idea in the statements of those who believe that diphtheria-like bacilli are simply transitory variations of the species *Bacillus diphtheriæ* is that both the diphtheria bacillus and those bacilli which resemble it have many unstable properties, their form, their cultural characteristics, their pathogenicity all varying within a wide limit, so that one form may assume readily the properties of another form.

The separatists, on the other hand, have found that certain forms possess such stable properties that one is not converted into another, and hence they regard them as distinct species.

In order to make a thorough test of this whole matter Williams[1] undertook a careful investigation of the subject which covered all the tests just described and many others.

The conclusions reached were as follows: Though some cultures change on some of the media, each changes in its own way, and each culture still has its distinct individuality. After many culture generations, especially when transplanted at short intervals, the different varieties of virulent diphtheria bacilli tend to run in lines parallel with a common norm, which seems to be a medium-sized, non-segmented bacillus producing granules in early cultures on serum and growing well on all of the ordinary culture media. The non-virulent morphologically typical bacilli must be classed with the virulent varieties as one species, though there is little doubt that more minute study would show that the former constitute a distinct group. The atypical pseudo forms, however, which show no tendency to approach the norm of the typical forms, must be classed as distinct species. All of the pseudo and the non-virulent morphologically typical varieties when inoculated into the peritoneum of guinea-pigs in immense doses cause death. Attempts have been made to give more virulence to some of these varieties by successive peritoneal inoculations, but in no instance has any increase of virulence or decided change in morphological or cultural characteristics been noted. Two of the non-virulent, morphologically typical varieties have also been grown in symbiosis with virulent streptococci in broth for ninety culture generations transplanted every three to four days, but when separated no change in virulence or other characteristics was noted. Two other varieties of non-virulent morphologically typical bacilli have been inoculated into goldfinches with no result. In large doses they appear to be perfectly innocuous to these birds as well as do four varieties of pseudo-bacilli, contrary to the results of Richmond and Salter.

Since there are so many different forms or varieties of diphtheria-like bacilli, it is quite possible that some of them are derived from strains of the diphtheria bacillus and that under certain conditions they readily regain its characteristics. This seems to be the only way

[1] Journal of Med. Research, June, 1902.

to explain the apparent discrepancies in the results obtained by different observers. Such closely related varieties, however, do not appear to be common in New York City at the present time. So we may safely say that in this region at least, non-virulent diphtheria-like organisms retain their characteristics under various artificial and natural conditions, and that they may be regarded from a public health standpoint as harmless.

Resistance to Heat, Drying, and Chemicals.—The thermal death point with ten minutes' exposure is about 60° C., with five minutes 70° C. Boiling kills in one minute. It has about the average resistance of non-spore-bearing bacteria to disintectants. In the dry state and exposed to diffuse light diphtheria bacilli usually die in a few days but they may live for months; when in the dark, or protected by a film of mucus or albumin, they may live for even longer periods. Thus we found scrapings from a dry bit of membrane to contain vigorous and virulent living bacilli for a period of four months after removal from the throat, and if the membrane had not been at that time completely used, living bacilli could probably have been obtained for a much longer period. On slate- and lead-pencils, toys, tumblers, as well as on paper money, they may live for several weeks, while on coins they die in twelve to thirty-six hours. In culture media, when kept at the blood heat, they usually die after a few weeks; but under certain conditions, as when sealed in tubes and protected from heat and light, they retain their virulence for years. The bacillus is not very sensitive to cold, for we found about 10 per cent. of the bacilli to retain their vitality and virulence after exposure for two hours to several hundred degrees below zero. At temperatures just below freezing they may remain alive for a number of weeks.

Transmission of Diphtheria.—The possibility of the transmission of diphtheria from animals to man cannot be disputed; we have met with two instances where cats had malignant diphtheria, and many other animals can be infected, but there are no authentic cases of such transmission on record. So-called diphtheritic disease in animals and birds is usually, if not always, due to other microörganisms than the diphtheria bacilli.

Let us consider some of the means by which the bacilli may be communicated. In actual experiment the bacilli have been observed to remain virulent in bits of dried membrane for twenty weeks. Dried on silk threads Abel reports that they may sometimes live one hundred and seventy-two days, and upon a child's plaything which had been kept in a dark place they lived for five months. The virulent bacilli have been found on soiled bedding or clothing of a diphtheria patient, or drinking-cups, candy, shoes, hair, slate-pencils, etc. Besides these sources of infection by which the disease may be indirectly transmitted, virulent bacilli may be directly received from the pseudomembrane, exudate, or discharges of diphtheria patients; from the secretions of the nose and throat of convalescent cases of diphtheria in which the virulent bacilli persist; and from the healthy throats of individuals

who acquired the bacilli from being in contact with others having virulent germs on their persons or clothing. In such cases the bacilli may sometimes live and develop for days or weeks in the throat without causing any lesion. When we consider that it is only the severe types of diphtheria that remain isolated during their actual illness, the wonder is not that so many, but that so few, persons contract the disease. It indicates that very frequently virulent bacilli are received into the mouth, and then either find no condition there suitable for their growth or are swept away by food or drink before they can effect a lodgment.

Susceptibility to and Immunity against Diphtheria.—An individual susceptibility, both general and local, to diphtheria, as in all infectious diseases, is necessary to contract the disease. Age has long been recognized to be an important factor in diphtheria. Children within the first six months of life are but little susceptible, but exceptionally infants of a few weeks are attacked, the greatest degree of susceptibility being between the third and tenth year. After that age susceptibility decreases. Young animals born of mothers immune to diphtheria possess nearly the same degree of immunity as their mothers. They gradually lose this but retain traces up to four to six months.

Diphtheria Antitoxin.—As the result of animal experiments, it is now known that an artificial immunity against diphtheria can be produced, by the action of toxin on the cells causing the development of substances directly antidotal to the diphtheria toxin. Behring, in conjunction with others, showed that the blood of immune animals contains a substance which neutralizes the diphtheria toxin. The blood serum of persons who have recovered from diphtheria has been found also to possess this protective property, which it acquires about a week after the beginning of the disease, and loses again in a few months. Moreover, the blood serum of many individuals, usually adults, who have never had diphtheria often has a slight general antitoxic property.

Neutralizing Characteristics of Antitoxin.—Diphtheria antitoxin has the power of neutralizing diphtheria toxin, so that when a certain amount is injected into an animal before or together with the toxin it overcomes its poisonous action. There is a direct action of antitoxins upon their corresponding toxins.

The various attempts to separate the toxins and antitoxins from neutral mixtures have been failures, and it is found that neutralization takes place according to the law of multiple proportions, *i. e.*, to save an animal from 1000 fatal doses of diphtheria toxin requires little more than a hundred times as much antitoxin as is required for ten fatal doses, the resistance of the animal itself accounting for the difference.

Nature of Diphtheria Antitoxin.—This has until recently been known almost wholly from its physiological properties. Experiments have seemed to show that it was either closely bound to the serum

globulins or was itself a substance of proteid nature closely allied to serum globulin. A fact developed by Atkinson is that the globulins tend to increase markedly in the serum of horses as the antitoxic strength increases. It seems possible from the above that diphtheria antitoxin has the characteristics of the serum globulins. Antitoxin is but little injured by prolonged moderate heat (56° C.) but is destroyed by short exposure to higher temperatures (95° to 100° C.). It is less sensitive than diphtheria toxin. Atkinson, when research chemist in our laboratory, found that in the case of antitoxic serum the globulin precipitate carries with it all of the antitoxic power of the serum, leaving the filtrate without any neutralizing power against the diphtheria toxin. Independently of Atkinson, Pick obtained similar results. These experiments were continued later by Gibson and Banzhaf and they proved that the globulins which were insoluble in saturated sodium chlorid solution carried with them no antitoxin. The soluble globulins which on heating become insoluble also contain no antitoxin. With this knowledge a practical method of eliminating much of the non-antitoxic portion of the serum was perfected.

Antitoxin Unit—Testing of Antitoxin.—This power, possessed by a definite quantity of antitoxin to neutralize a certain amount of toxin, is utilized in testing the amount of antitoxin in any serum or solution. We measure this amount in units. A unit may be defined as the amount of antitoxin which will just neutralize 100 minimal fatal doses of toxin for a 250-gram guinea-pig.

There are certain peculiarities in the composition of toxins which require us to use certain precautions in selecting the one to be used for testing. This we call a standard toxin.

In order to facilitate testing we consider that a guinea-pig which lives after injection more than four days, is protected. The test is carried out as follows: Guinea-pigs of about 250 grams' weight are subcutaneously injected with one hundred fatal doses of a carefully preserved standardized toxin, which toxin has been previously mixed with an amount of antitoxin believed to be sufficient to protect the animal. If the guinea-pig lives four days, even if it becomes seriously ill, the amount of antitoxin added to the one hundred fatal doses of toxin is considered to have neutralized it and to measure 1 unit. If the guinea-pig dies earlier than four days, less than 1 unit of antitoxin was in the mixture.

Production of Diphtheria Antitoxin for Therapeutic Purposes.—As a result of the work of years in the laboratories of the Health Department of New York City, the following may be laid down as a practical method:

A strong diphtheria toxin should be obtained by taking a very virulent culture and growing it in broth under the conditions described on page 203.

The horses used should be young, vigorous, of fair size, and absolutely healthy. The horses are severally injected with 10000 units of

antitoxin and with toxin[1] sufficient to kill five thousand guinea-pigs of 250 grams' weight. After from three to five days, so soon as the fever reaction has subsided, a second subcutaneous injection of a slightly larger dose is given. The following figures give the actual injections in a horse which produced an unusually high grade of serum.

Actual Injections in Horse.—Injections of toxin were given every three days in the following amounts:

First day, 12 c.c. toxin ($\frac{1}{400}$ c.c. fatal dose), together with 10000 units of diphtheria antitoxin.

Second and later injections of toxin without antitoxin at three-day intervals as follows: 15 c.c., 45 c.c., 55 c.c., 65 c.c., 80 c.c., 95 c.c., 115 c.c., 140 c.c., (twenty-eighth day), 170 c.c., 205 c.c., 250 c.c., 300 c.c. (fortieth day). The injections were gradually increased until on the sixtieth day, 675 c.c. were given.

The antitoxic strength of the serum was on the twenty-eighth day, 225 units; on the fortieth day, 850 units; and on the sixtieth day, 1000 units. Regular bleedings were made weekly for the next four months when the serum had fallen to 600 units in spite of weekly, gradually increasing doses of toxin.

If the antitoxin is not given we begin with .02 c.c. of toxin.

There is absolutely no way of judging which horses will produce the highest grades of antitoxin. Very roughly, those horses which are extremely sensitive and those which react hardly at all are the poorest, but even here there are exceptions. The only way, therefore, is at the end of six weeks or two months to bleed the horses and test their serum. If only high-grade serum is wanted all horses that give less than 150 units per c.c. are discarded. The retained horses receive steadily increasing doses, the rapidity of the increase and the interval of time between the doses (three days to one week) depending somewhat on the reaction following the injection, an elevation of temperature of more than 3° F. being undesirable. At the end of three months the antitoxic serum of all the horses should contain over 300 units, and in about 10 per cent. as much as 800 units in each cubic centimetre. Not more than 1 per cent. give above 1000 units, and none so far has given as much as 2000 units per c.c. The very best horses if pushed to their limit continue to furnish blood containing the maximum amount of antitoxin for several months, and then, in spite of increasing injections of toxin, begin to furnish blood of gradually decreasing strength. If every nine months an interval of three months' freedom from inoculations is given, the best horses furnish high-grade serum during their periods of treatment for from two to four years.

[1] The culture, after a week's growth, is removed, and having been tested for purity by microscopic and culture tests is rendered sterile by the addition of 10 per cent. of a 5 per cent. solution of carbolic acid. After forty-eight hours the dead bacilli have settled on the bottom of the jar and the clear fluid above is siphoned off, filtered, and stored in full bottles in a cold place until needed. Its strength is then tested by giving a series of guinea-pigs carefully measured amounts. Less than 0.005 c.c., when injected hypodermically, should kill a 250-gram guninea-pig.

In order to obtain the serum the blood is withdrawn from the jugular vein by means of a sharp-pointed cannula, which is plunged through the vein wall, a slit having been made in the skin. The blood is carried by a sterile rubber tube into large Erlenmeyer flasks, held slanted or into cylindrical jars, and allowed to clot. The serum is drawn off after four days by means of sterile glass and rubber tubing, and is stored in large flasks. Instead of this process when the globulins are to be separated the blood may be added directly to one-tenth of its volume of a 10 per cent. solution of sodium citrate. This prevents clotting of the blood. With the serum or globulin solution, small phials are filled. The phials and their stoppers, as indeed all the utensils used for holding the serum, must be absolutely sterile, and every possible precaution must be taken to avoid contamination. An antiseptic may be added to the serum as a preservative, but it is not necessary except when the serum is to be sent to great distances, where it cannot be kept under supervision.

Kept from access of air and light and in a cold place it is fairly stable, deteriorating not more than 30 per cent., and often much less, within a year. Diphtheria antitoxin, when stored in phials and kept under the above conditions, contains within 10 per cent. of its original strength for at least two months; after that it can be used by allowing for a maximum deterioration of 2 per cent. for each month. The antitoxin in old serum is just as effective as in that freshly bottled, only there is less of it. The serum itself is less apt to produce rashes. All producers put more units in the phials than the label calls for, so as to allow for gradual loss of strength.

Technical Points upon the Testing of Diphtheria Antitoxin and the Relations between the Toxicity and Neutralizing Value of Diphtheria Toxin.—During the earlier investigations the filtered or sterilized bouillon, in which the diphtheria bacillus had grown and produced its "toxin," was supposed to require for its neutralization an amount of antitoxin directly proportional to its toxicity as tested in guinea-pigs. Thus, if from one bouillon culture ten fatal doses of "toxin" were required to neutralize a certain quantity of antitoxin, it was believed that ten fatal doses from every culture, without regard to the way in which it had been produced or preserved, would also neutralize the same amount of antitoxin. Upon this belief was founded the original Behring-Ehrlich definition of an antitoxin unit that it was ten times the amount of antitoxin which neutralized ten fatal doses of toxin.

The results of tests by different experimenters with the same antitoxic serum, but with different diphtheria toxins, proved this opinion to be incorrect. Ehrlich[1] deserves the credit for first clearly perceiving and publishing this. He obtained from various sources twelve toxins and compared their neutralizing value upon antitoxin; these tests gave most interesting and important information. The results in four

[1] Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen. Klinisches Jahrbuch, 1897.

toxins, which are representative of the twelve, are as shown in the following table:

Toxin specimen number of Ehrlich.	Estimated "minimal" fatal dose for 250-gm. guinea-pigs.	Smallest number of fatal doses of toxic bouillon required to kill a 250-gm. guinea-pig within 5 days, when mixed with one antitoxin unit, "L_+ Ehrlich."	Fatal doses required to "completely neutralize one antitoxin unit" as determined by the health of the guinea-pig remaining unaffected "L_0" Ehrlich	$L_+ - L_0$ = fatal doses.	Data upon "toxin" specimen given by Ehrlich.
4	0.009	39.4	33.4	6	Old, deteriorated from 0.003 to 0.009.
7	0.0165	76.3	54.4	22	Fresh toxin, preserved with tricresol.
9	0.039	123	108	15	A number of fresh cultures grown at 37° C. 4 and 8 days.
12	0.0025	100	50	50	Tested immediately after its withdrawal.

From the facts set forth in the table, Ehrlich believed that the diphtheria bacilli in their growth produce a toxin which, so long as it remains chemically unaltered, has a definite poisonous strength with a definite value in neutralizing antitoxin. The toxin is, however, an unstable compound, and begins to change almost immediately into substances which are not, at least acutely, poisonous, but which retain their power to neutralize antitoxin.

The results of some experiments of Atkinson and Park[1] were fully in accord with those published by Ehrlich as to the varying neutralizing value of a minimal fatal dose of "toxin"; they, however, also indicate roughly a general law in accordance with which these changes occur.

The neutralizing value of a fatal dose of toxin is at its lowest in the culture fluid when the first considerable amounts of toxin have been produced. After a short period, during which the quantity of toxin in the fluid is increasing, the neutralizing value of the fatal dose begins to increase, at first rapidly, then more slowly.

While the culture is still in vigorous growth and new toxin is being produced, the neutralizing value of the fatal dose fluctuates somewhat, but with a generally upward tendency. After the cessation of toxin production the neutralizing value of the fatal dose increases steadily until it becomes five to ten times its original amount.

In our experiments the greatest value for L_+ was 126, the least 27. As at six hours L_+ was only 72 and at twenty-eight hours only 91, we doubt whether L_+ ever reaches above 150.[2] When we seek to analyze the above-

L_0 = fatal doses of toxin required to fully neutralize one unit of antitoxin.

described process we find certain facts which seem partly to explain it.

In the fluid holding the living bacilli we have, after the first few hours of toxin formation, a double process going on—one of deterioration in the toxin already accumulated, which tends to increase the neutralizing value of the

[1] Journal of Experimental Medicine, Vol. iii, No. 4.

[2] L_+ = fatal doses of toxin required to kill a guinea-pig in four days after having been mixed with one unit of antitoxin.

fatal dose; the other of new toxin formation, which probably tends to diminish the neutralizing value. The chemical changes produced by the growth of the bacilli in the bouillon tend to aid one or the other of these processes, and so to make, from hour to hour, slight changes in the value of the fatal dose. Later, with the period of cessation of toxin production, the gradual deterioration of the toxicity alone continues, and the fatal dose gradually and steadily increases in its neutralizing value. We believed that two types of toxin were produced by the bacilli.

With greater information Ehrlich has had to modify greatly the details of his earliest explanation of the reason of the variation in the ratio between toxicity and neutralizing value of toxin. He now accepts the fact that diphtheria culture fluid contains at least two toxins which differ in their characteristics.

To summarize Ehrlich's present views as to the nature of diphtheria toxin: The diphtheria bacillus secretes two toxins, one of which, the toxin, causes the acute phenomena of diphtheria intoxication, while the other, the toxon, causes cachexia and paralysis after a rather long period of incubation. The non-toxic toxin, or toxoid, appears as the result of the degeneration of the toxophore group of the toxin, the haptophore group remaining intact. The toxin may be separated into three divisions, which vary in their affinity for antitoxin—prototoxin, deuterotoxin, and tritotoxin. On the same basis there are three toxoids—prototoxoids, syntoxoids, and epitoxoid (the toxon) —the first having the greatest affinity for antitoxin, while the epitoxoid has the least. The toxins are divided into an alpha and a beta portion, depending on the ease with which they are changed into toxoids. All of these substances unite with tissue cells and with antitoxin through the agency of a haptophore group, while the toxicity depends on the presence of a toxophore group in the toxin or toxon molecule.

Bordet and others refuse to accept these complicated conceptions of Ehrlich and the whole matter is at the present time under active discussion. Thus the existence or non-existence of toxons has excited a great deal of discussion among investigators. The Swedish chemist, Arrhenius, has quite recently given much attention to toxons and is applying the principles of physical chemistry to the study of toxins and antitoxins. It is a well-known fact that some chemical substances when in solution have the power of breaking up into their constituent parts; thus sodium chloride breaks up in part into sodium and chlorine, as sodium or chlorine ions or electrolytes. The dissociated sodium and chlorine may then enter into combination with any other suitable substance which may be present. Arrhenius holds that this is the case with the toxin-antitoxin molecule, that it may to a certain extent again break up into separate toxin and antitoxin. He believes that this dissociated toxin is the substance which Ehrlich has been calling toxon. Madsen, who formerly had done much work with toxons, has now joined with Arrhenius in support of the dissociation theory. In spite of their reasoning Ehrlich and his followers continue to uphold the toxon as an independent toxic substance. Recent investigations throw doubt on both explanations as being at all final.

Standardizing of Antitoxin Testing.—Ehrlich has contributed greatly to uniformity of results in testing antitoxin by calling attention to the necessity of selecting a suitable toxin and by employing and distributing an antitoxin as a standard to test toxins by. In this way smaller testing stations can make their results correspond with those of the central station. The United States Marine Hospital laboratory has also distributed to laboratories in the United States an equally carefully standardized serum.

The old definition of Behring and Ehrlich, that an antitoxin unit

contains the amount of antitoxin which will protect the life of a guinea-pig from one hundred fatal doses of toxin, is true only for a toxin similar to that adopted as the standard—namely, one having approximately the characteristics of toxins in cultures at the height of their toxicity.

The actual test of an antitoxin serum is, therefore, carried out as follows: Six guinea-pigs are injected with mixtures of toxin and antitoxin. In each of the mixtures there is the amount of toxin sufficient to just neutralize 1 unit of the standard antitoxin supplied by the central laboratory. In each of the mixtures the amount of antitoxin varies; for instance, No. 1 would contain 0.002 c.c. serum; No. 2, 0.003 c.c.; No. 3, 0.004 c.c.; No. 4, 0.005 c.c., etc. If at the end of the fourth day Nos. 1, 2, and 3 were dead and Nos. 4, 5, and 6 were alive we would consider the serum to contain 200 units units of antitoxin for each cubic centimetre. When we test for experimental purposes sera with very little antitoxin, we often use only one-tenth the above amount of toxin. In this case the resistance of the guinea-pig must be considered so that the guinea-pig must not only not die but must remain well. The mixed toxin and antitoxin must remain together for fifteen minutes before injecting so that complete union may occur.

Use of Antitoxin in Treatment and Immunization.—The antitoxin in the higher grades of globulin solution or serum is identical with that in the lower grades; there is simply more of it in each drop. In treatment, however, for the same amount of antitoxin we have to inject less foreign proteids with the higher grades, and, therefore, have somewhat less danger of rashes and other deleterious results. The amount of antitoxin required for immunization is 300 to 500 units for an infant, 500 to 1000 for an adult, and proportionately for those between these extremes. The larger doses are advised when the danger of infection is very great. After the observation of the use of antitoxin in the immunization of several thousand cases, I have absolute belief in its power to prevent an outbreak of diphtheria for at least two weeks, and also of its almost complete harmlessness in the small doses required. If it is desired to prolong the immunity the antitoxin injection is repeated every two weeks. For treatment, mild cases should be given 1500 units, moderate cases 2000 to 4000 units, and severe cases 10,000 to 20,000 units. Where no improvement follows in twelve hours the dose should be repeated. Intravenous injections give most rapid effect, and should be used in all malignant cases. It takes twelve to eighteen hours for the absorption into the blood of the greater part of the antitoxin from the subcutaneous tissues. This in bad cases may be a fatal delay. Antitoxin is only to a very slight extent absorbed when given by the mouth.

Results of the Antitoxin Treatment of Diphtheria.—The conclusions arrived at by Biggs and Guerard, after a review of all the statistics and opinions published since the beginning of the antitoxin treatment in 1892, were as follows:

"It matters not from what point of view the subject is regarded, if the evidence now at hand is properly weighed, but one conclusion is or can be reached—whether we consider the percentage of mortality from diphtheria and croup in cities as a whole, or in hospitals, or in private practice; or whether we take the absolute mortality for all the cities of Germany whose population is over 15,000, and all the cities of France whose population is over 20,000; or the absolute mortality for New York City, or for the great hospitals in France, Germany, and Austria; or whether we consider only the most fatal cases of diphtheria, the laryngeal and operative cases; or whether we study the question with relation to the day of the disease on which treatment is commenced, or the age of the patient treated; it matters not how the subject is regarded or how it is turned for the purpose of comparison with previous results, the conclusion reached is always the same —namely, there has been an average reduction of mortality from the use of antitoxin in the treatment of diphtheria of not less than 50 per cent., and under the most favorable conditions a reduction to one-quarter, or even less, of the previous death rate. This has occurred not in one city at one particular time, but in many cities, in different countries, at different seasons of the year, and always in conjunction with the introduction of antitoxin serum and proportionate to the extent of its use." The combined statistics of deaths of 19 of the chief cities of the world show there were in the last four years not over 30 per cent. as many deaths as in the four years preceding the introduction of antitoxin. Except where immunization has been practiced on a large scale, no marked reduction in the number of cases of diphtheria has been evident.

Deleterious Effects Following Injections of Antitoxic Serum.—About 1 in 10,000 persons develop, within a few minutes after an injection of serum, alarming symptoms. About twenty deaths in all have been reported. The persons suffering severe symptoms have usually been subject to asthma while the fatal cases usually have the pathological changes known as status lymphaticus. A few of these rare cases die almost instantly. As a rule, when death occurs it takes place within a few minutes after the development of symptoms. Usually the respiratory rather than the circulatory center seems to be affected. It is over three years since a death from this cause has occurred in New York City.

Serum Sickness.—Besides these rare accidents there are the disagreeable after-effects which we group under the name serum sickness. Under this name we now include the various clinical manifestations following the injection of horse serum into man. The principal symptoms of this disease are a period of incubation varying from eight to thirteen days, fever, skin eruptions, swelling of the lymph glands, leukonemia, joint symptoms, œdema, and albuminuria. The term "serum sickness" was first used by von Pirquet and Schick,[1] from whose excellent monograph the following data are chiefly taken.

[1] v. Pirquet and Schick, Die Serum Krankheit, Wien, 1905.

In 1874 Dallera reported that urticarial eruptions may follow the transfusion of blood. In the year 1894 the use of diphtheria antitoxin introduced the widespread practice of injecting horse serum. In the same year several cases were reported in which these injections were followed by various skin manifestations, mostly of an urticarial character. Following these came a great mass of evidence which made it clear that following the injection of antidiphtheric serum these sequelæ were usually comparatively harmless.

DUE TO SERUM AS SUCH.—Heubner in 1894 and von Bokay somewhat later expressed the opinion that these manifestations were due to other properties than the antitoxin in the serum, and this has proven to be the case. It has also been shown that the skin eruptions and other symptoms follow in a considerable degree according to the amount of serum injected, and this has led to attempts to eliminate the non-antitoxic portion of the serum as much as possible.[1] The serum reaction has been studied by many investigators, but is not yet fully understood.

VON PIRQUET AND SCHICK'S SENSITIZATION THEORY (ANAPHYLAXIS). —The underlying idea is that the injection of serum into animals causes the development of specific reaction products which are able to act upon the antigens introduced. These antibodies encounter the antigens, *i.e.*, the serum later introduced in the body, and so give rise to a deleterious reaction. This accounts for the cases of "immediate reaction" in man described by von Pirquet and Schick, in which second injection of a serum produces an attack of serum sickness with a short or no period of incubation. It has been known for years that a large injection of horse serum is poisonous to guinea-pigs, that have been previously injected with small amounts of horse serum.[2] The time necessary to elapse between the first and second injections is ten days or more. The greatest effect is present between four and six weeks. The symptoms are respiratory embarrassment, paralysis and convulsions, and come on usually within ten minutes after the injection. When death results it usually occurs within one hour, frequently in less than thirty minutes. The poisonous principle in horse serum in these cases appears to act on the respiratory centers. The heart continues to beat long after respiration ceases.

The first injection of horse serum renders the guinea-pig susceptible; the quantity required for this purpose is extremely small. Rosenau and Anderson find that from $\frac{1}{250}$ to $\frac{1}{1000}$ c.c. ordinarily suffices. Guinea-pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat.

It is probable that man cannot be sensitized in the same way as guinea-pigs, the most susceptible of the laboratory animals. Children have, in numerous instances, been injected with antidiphtheric horse

[1] See Gibson, The Concentration of Diphtheria Antitoxin. Jour. of Biological Chemistry, Vol. i, 1906.

[2] The Germans usually speak of this as "Theobald Smith's phenomenon of hypersusceptibility" (see p. 162).

serum at short and long intervals without, so far as we are aware, causing severe symptoms. Certain serums, for example the antitubercle serum of Maragliano, are habitually used by giving injections at intervals of days or weeks. The rare fatal cases so far reported have all followed primary injections.

While it may be true that the sensitizing of guinea-pigs by a previous injection of serum is analogous to the condition present in man which gives rise to the sudden symptoms following an injection of antitoxic serum, there is, in our experience, no reason to avoid a second immunizing injection of serum when it is really indicated. A subcutaneous injection in man comparable to the amount required to produce sickness in a guinea-pig would be over 200 c.c. We should hesitate, however, to give a large intravenous injection in a sensitized child. Banzhaf and Famuleuer have recently shown that chloral in large doses will prevent sickness in sensitized guinea-pigs.

The Separation of Antitoxin from Serum.—There have already been many attempts to accomplish this in the case of the antitoxins. Those interested in the chemical side of these investigations are referred to the recent article by Gibson, as already stated. In 1900, Atkinson, working in the Research Laboratory of the Health Department, eliminated all but the globulin from the antitoxic serum, and we tried this partially refined serum in 36 cases. The results were so nearly identical with an equal number of cases treated with the whole serum from the same horse that it did not seem to be worth while to go to the expense of preparing such an antitoxic solution. The idea that a practical separation of the antitoxin from much of the proteid non-antitoxic portion of the serum was possible was not given up. In August of 1905 we began trials with an antitoxic preparation which offered grounds for hoping for better success. Dr. R. B. Gibson, chemist in the Research Laboratory, placed the ammonium sulphate precipitate from the antitoxic serum in saturated sodium chloride solution and found that the portion of the globulin soluble in this contained all the antitoxin. In this way the nucleoproteids and the insoluble globulins present in the Atkinson preparation were eliminated, as in the following summary shows.

Ordinary antitoxic serum contains serum globulins (antitoxic), serum globulins (non-antitoxic), serum albumins (non-antitoxic), serum nucleoproteids (non-antitoxic), cholesterin, lecithin, traces of bile coloring matter, traces of bile salts and acids, traces of inorganic blood salts and other non-proteid compounds. Refined serum contains serum globulins (antitoxic), traces of serum globulins (non-antitoxic), dissolved in dilute saline solution. Later Dr. E. A. Banzhaf,[1] who had succeeded Gibson, discovered that if the antitoxic serum or plasma was heated to 57° for 18 hours there was a change of a considerable portion of the soluble globulins into insoluble globulins. The antitoxin remained unchanged. This permitted a greater elimination of the non-antitoxic proteids.

[1] Journal of Biological Chemistry.

METHOD OF CONCENTRATION.—The material we use is blood plasma instead of blood serum. This is obtained by allowing the blood to flow directly from the jugular vein of the immunized horse into 10 per cent. sodium citrate solution, which prevents it from clotting and allows the red corpuscles to settle out. This plasma is used, in place of serum, merely as a matter of convenience and economy.

ISOLATING THE ANTITOXIN GLOBULINS.—The globulins of the plasma are removed from the other constituents by precipitating them from solution by means of ammonium sulphate and filtering off on paper. This allows the serum albumins and other soluble, non-proteid constituents of the blood, to pass through and thus become immediately eliminated in the filtrate. Now this precipitate, formed with ammonium sulphate, contains the globulins of the blood which are antitoxic in character; those which are non-antitoxic in character; and nucleoproteids. The antitoxic globulins are extracted from this mass of precipitate by treating with saturated solution of sodium chloride, in which this compound is soluble. The problem then remains to separate this antitoxic substance from the solution and wash out of it the salts of ammonium and sodium.

The antitoxic globulin is next isolated by precipitation with dilute acetic acid.

The ammonium salts are thoroughly washed out by repeated treatment with saturated sodium chloride solution and filtered each time. Finally, the sodium chloride is removed by dialysis, which process is accomplished by placing the antitoxic globulins in bags of vegetable parchment and immersing in running water so long as salts continue to diffuse out. After dialysis, the antitoxic globulins are dissolved in dilute saline solution, filtered through paper pulp, to remove the traces of undissolved matter, filtered through a Berkefeld clay filter to remove bacteria, and then put in sterile syringes.

This antitoxic solution of globulin and a portion of the other soluble serum globulins was then tested on a number of children. The results were from the start favorable, except that in the beginning more local pain was produced than with the whole serum. Stricter attention to the neutralization soon overcame this, so that when the serum was injected on one side and the globulin solution on the other the patient was unable to tell one from the other. In October, 1905, the antitoxic globulin solution was administered not only in the hospitals, but also in private homes by medical inspectors. Since then it has been the only form of antitoxin supplied by the Health Department. Private manufacturers have also recently begun to furnish it.

Results from the Use of Antitoxic Globulin Solution.—The curative effect proved to be identical with that of the whole serum. Our tests showed clearly that not only the toxin, but also the poisons produced in the animal by injections with virulent bacilli are neutralized as completely by the globulin solution as by the antitoxic serum from which they are separated. Not only we ourselves, but the resident and attending physicians of the contagious disease hospitals noted that following

the injections of the globulin solution there seemed to be decidedly less severe rashes than formerly followed the whole serum, and it was especially noted that there were very few who had any constitutional disturbances even when the development of the rashes did occur. As the serum supplied by different horses or from the same horse at different times is known to vary, and as it is therefore difficult accurately to compare different bleedings, it was decided to make a test by collecting a quantity of serum from four different horses, mixing it thoroughly, and then, after precipitating one-half, to treat an equal number simultaneously with the two preparations. These tests were chiefly carried out in the Willard Parker Hospital, but a few of the cases were treated at Riverside Hospital. It soon became evident that the serum that we had chosen for the test was one of such character that eruptions and constitutional disturbances usually appeared in the children injected. In those over ten years of age almost no rashes occurred. The rashes in those given the globulin preparation were much less severe. The cases treated with both the whole serum and the antitoxic globulins were most carefully watched, and the course of the disease as well as after-affects noted.

After all the tested cases had become fully convalescent or had left the hospital, the histories were finally gone over and compared. It was found that fifty children under ten years of age treated with the whole serum had lived at least nine days or long enough for the development of serum effects. The first fifty consecutive cases in children under ten years treated with the antitoxic globulins precipitated from the same lot of serum were taken to compare with these.

The comparative table giving a summary of the constitutional and local reactions obtained in the treatment of fifty cases of diphtheria in young children, with a lot of antitoxic serum received from three horses and of an equal number of similar cases treated with a solution of the antitoxic globulins derived from a portion of the same lot of serum is as follows:

	Children treated with the whole serum	Children who were treated with the antitoxic gobulins
Marked constitutional symptoms accompanied by a severe and persistent rash in	28 per cent.	0 per cent.
Moderate constitutional symptoms accompanied by a well-developed erythema or urticaria	18 per cent.	4 per cent.
Very slight constitutional disturbance accompanied by a more or less general rash in	20 per cent.	8 per cent.
No appreciable constitutional disturbance, but more or less general urticaria or erythema in	4 per cent.	34 per cent.
No appreciable deleterious after-effects whatever in	30 per cent.	54 per cent.

DURATION OF RASHES.

Days	1	2	3	4	5	6	7	8	Totals
Antitoxic globulin cases	5	7	5	2	3		1		—23
Whole serum cases	1	4	10	1	10	3	2	5	—36

The concentration of antitoxin made possible by the elimination of the non-antitoxic substances is not only a convenience but is of a distinct importance, as it tends to encourage large doses. Some producers supply a product which is too rich in proteid. This is probably not so well absorbed as the less concentrated product. The total solids in the globulin solution should not be much greater than those in the serum.

The antitoxic globulin solution tends to become slightly cloudy when kept at moderate or high temperatures and substances such as solutions of carbolic acid and tricresol precipitate it.

Development of Agglutinins for Diphtheria Bacilli.—By the injections of the bodies of diphtheria bacilli into animals agglutinins have been developed in sufficient amount to act in 1:5000 dilutions of the serum. The serum produced from diphtheria bacilli does not agglutinate pseudodiphtheria bacilli in high dilutions. The serum of patients convalescent from diphtheria has, as a rule, little agglutinating power. This test is not used in diagnosis.

Persistence of Antitoxin in the Blood.—When injections of toxin are stopped in a horse the antitoxin is slowly eliminated, so that there is a loss of about 20 per cent. a week. In from five to eight months all appreciable antitoxin has been eliminated.

The Persistence in the Man's Blood of Injected Antitoxin Produced in the Horse.—All observers from Ransom on, except Madsen and Roemer, have noted that antitoxins and other antibodies produced in an animal disappear more rapidly when introduced into the blood of another species than into one of the same species.

Madsen and Roemer claim that the antibodies from each species must be tested in other species and that, in some cases, the foreign antibody will persist as long as that obtained from one of the same species.

When their experiments are examined it is seen that Madsen's results were obtained in four animals only, and that one of the goats receiving its own type of antitoxin died on the seventeenth day. Roemer's recorded observations really substantiate the claims made by other investigators, for when he injected lambs with heterologous antitoxin it disappeared just as rapidly as in the animals tested by others. He tested for such very slight amounts of antitoxin, however, that it appeared to last longer than in the animals of others, who did not test for such small amounts.

In our experiments in guinea-pigs we have found that the homologous antitoxin was retained in appreciable amounts for at least six months, while the heterologous antibodies were noticeable to the same extent for only four weeks. There is a very rapid loss of both types of antitoxins during the first two weeks and then a slow loss becoming more and more gradual until final elimination. The larger the amount of antibodies injected the longer will be the time before the elimination of effective amount.

Active Immunization.—Theobald Smith has recommended that mixtures of toxin and antitoxin be given so as to produce active immunity. It is a well-known fact that when 60% of the L + dose of toxin is added to one unit of antitoxin, that this mixture will cause the production in the animal of antitoxin, and as a rule cause no toxic symptoms. The immunity produced from a single injection is slight, but will last for from nine to twelve months. Some guinea-pigs, however, some weeks after the injection, show a late paralysis and it is questionable whether we would dare to give such mixtures to children. Another practical objection is that the immunity during the first two weeks after the injection is almost negligible. Usually this is the period during which we desire the greatest immunity, because it is then that the danger of infection is greatest.

Mixed Infection in Diphtheria.—Virulent diphtheria bacilli are not the only bacteria present in human diphtheria. Various cocci, more particularly streptococci, staphylococci, and pneumococci, are also found actively associated with Loeffler's bacilli in diphtheria, playing an important part in the disease and leading often to serious complications (sepsis and bronchopneumonia). Though the results of these investigations so far have been somewhat indefinite, they would seem to indicate that when other bacteria are associated with the diphtheria bacilli they mutually assist one another in their attacks upon the mucous membrane, the streptococcus being particularly active in this respect, often opening the way for the invasion of the Loeffler bacillus into the deeper tissues or supplying needed conditions for the development of its toxin. In most fatal cases of bronchopneumonia following laryngeal diphtheria we find not only abundant pneumococci or streptococci in the inflamed lung areas, but also in the blood and tissues of the organs. As these septic infections due to the pyogenic cocci are in no way influenced by the diphtheria antitoxin, they frequently are the cause of the fatal termination. Other bacteria cause putrefactive changes in the exudate, producing alterations in color and offensive odors.

Pseudomembranous Exudative Inflammations Due to Bacteria other than the Diphtheria Bacilli.—The diphtheria bacillus, though the most usual, is not the only microörganism that is capable of producing pseudomembranous inflammations. There are numerous bacteria present almost constantly in the throat secretions, which, under certain conditions, can cause local lesions very similar to those in the less-marked cases of true diphtheria. The streptococcus and pneumococcus are the two forms most frequently found in these cases, but there are also others, such as the Vincent's bacillus, which, under suitable conditions, excite this form of inflammation, but without constitutional symptoms.

The pseudomembranous angina accompanying scarlet fever, and to a less extent other diseases, may not show the presence of diphtheria bacilli, but only the pyogenic cocci, especially streptococci, or, more rarely, some varieties of little-known bacilli. The deposit cover-

ing the inflamed tissues in these non-specific cases is, it is true, usually but not always, rather an exudate than a true pseudomembrane.

Relation of Bacteriology to Diagnosis.—We believe that all experienced clinicians will agree that, when left to judge solely by the appearance and symptoms of a case, there are certain mild exudative inflammations of the throat which are at times excited by diphtheria bacilli and at times by other bacteria.

It is not meant to imply that a case is one of true diphtheria simply because the diphtheria bacilli are present, but rather that the doubtful cases not only have the diphtheria bacilli in the exudate, but are capable of giving true characteristic diphtheria to others, or later develop it characteristically themselves; and that those in whose throats no diphtheria bacilli exist can under no condition give true characteristic diphtheria to others, or develop it themselves unless they receive a new infection. It is, indeed, true, as a rule, that cases presenting the appearance of ordinary follicular tonsillitis in adults are not due to the diphtheria bacillus. On the other hand, in small children mild diphtheria very frequently occurs with the semblance of rather severe ordinary follicular tonsillitis, due to the pyogenic cocci, and in large cities where diphtheria is prevalent all such cases must be watched as being more or less suspicious. As showing doubt in our judgment, I think most would feel that if in any case exposure to diphtheria is known to have occurred, even a slightly suspicious sore throat would be regarded as probably due to the diphtheria bacilli. If, on the other hand, no cases of diphtheria have been known to exist in the neighborhood, even cases of a more suspicious nature would probably not be regarded as diphtheria.

Appearances Characteristic of Diphtheria.—The presence of irregular-shaped patches of adherent grayish or yellowish-gray pseudomembrane on some other portions than the tonsils is, as a rule, an indication of the activity of the diphtheria bacilli. Restricted to the tonsils alone, their presence is less certain.

Occasionally, in scarlatinal angina or in severe phlegmonous sore throats, patches of exudate may appear on the uvula or borders of the faucial pillars, and still the case may not be due to the diphtheria bacilli; these are, however, exceptional. Thick, grayish pseudomembranes which cover large portions of the tonsils, soft palate, and nostrils are almost invariably the lesions produced by diphtheria bacilli.

The very great majority of cases of pseudomembranous or exudative laryngitis, in the coast cities at least, whether an exudate is present in the pharynx or not, are due to the diphtheria bacilli. Nearly all membranous affections of the nose are true diphtheria. When the membrane is limited to the nose the symptoms are, as a rule, very slight; but when the nasopharynx is involved the symptoms are usually grave.

Most cases of pseudomembranes and exudates, entirely confined to portions of the tonsils in adults, are not due to the diphtheria bacilli. Cases presenting the appearances found in scarlet fever, in which a thin, grayish membrane lines the borders of the uvula and faucial

pillars, are rarely diphtheritic. As a rule, pseudomembranous inflammations complicating scarlet fever, syphilis, and other infectious diseases are due to the activity of the pathogenic cocci and other bacteria, induced by the inflamed condition of the mucous membranes due to the scarlatinal or other poison.

Location of Diphtheritic Inflammation.—Diphtheria attacks not only the fauces, larynx, and nasal cavities, but also occasionally the skin, vagina, rectum, conjunctiva, nose, and ear.

Exudate Due to the Diphtheria Bacilli Contrasted with that Due to Other Bacteria.—As a rule, the exudate in diphtheria is firmly incorporated with the underlying mucous membrane, and cannot be removed without leaving a bleeding surface, at least until convalescence. The tissues surrounding the exudate are more or less inflamed and swollen. Where other bacteria produce the irritant the exudate, except in cases due to the bacillus described by Vincent, is usually loosely attached, collected in small masses, and easily removable. Exceptions, however, occur in both these diseases, so that in true diphtheria the exudate may be easily removed, and in lesions due to other bacteria the exudate may be firmly adherent.

Paralysis following a pseudomembranous inflammation is an almost positive indication that the case was one of diphtheria, although slight paralysis has followed in a very few cases in which careful cultures revealed no diphtheria bacilli. These, if not true diphtheria, must be considered very exceptional cases.

Bacteriologic Diagnosis.—From the above it is apparent that fully developed characteristic cases of diphtheria are readily diagnosticated, but that many of the less marked, or at an early period undeveloped, cases are difficult to differentiate the one from the other. In these cases cultures are of the utmost value, since they enable us to isolate those in which diphtheria-like bacilli are found, and to give preventive injections of antitoxin to both the sick and those in contact with them, if this has not already been done. As a rule, cultures do not give us as much information as to the gravity of the case as the clinical appearances, for by the end of twenty-four to forty-eight hours the extent of the disease is usually possible of determination. The reported absence of bacilli in a culture must be given weight in proportion to the skill with which the culture was made, the suitableness of the media, the location of the disease, and the knowledge and experience of the one who examined it.

Diphtheria does not occur without the presence of the diphtheria bacilli; but there have been many cases of diphtheria in which, for one or another reason, no bacilli were found in the cultures by the examiner. In many of these cases later cultures revealed them. The reverse is also true, the presence of diphtheria bacilli in throats without the clinical signs of diphtheria in no sense makes it a case of diphtheria. In a convalescent case the absence of bacilli in any one culture indicates that there are certainly not many bacilli left in the throat. Only repeated cultures can prove their total absence.

Technique of the Bacteriologic Diagnosis.—*Collection of the Blood Serum and its Preparation for Use in Cultures.*—A covered glass jar, which has been thoroughly cleansed with hot water, is taken to the slaughter-house and filled with freshly shed blood from a calf or sheep. The blood is received directly in the jar as it spurts from the cut in the throat of the animal. After the edge of the jar has been wiped it is covered with the lid and set aside, where it may stand quietly until the blood has thoroughly clotted. The jar is then carried to the laboratory and placed in an ice-chest. If the jar containing the blood is carried about before the latter has clotted, very imperfect separation of the serum will take place. It is well to inspect the blood in the jar after it has been standing a few hours, and, if the clot is found adhering to the sides, to separate it by a rod. The blood is allowed to remain twenty-four hours on the ice, and then the serum which surrounds the clot is siphoned off by a rubber tube and mixed with one-third its quantity of nutrient beef-broth, to which 1 per cent. glucose has been added. This constitutes the Loeffler blood-serum mixture. This is poured into tubes, which should be about four inches in length and one-half of an inch in diameter, having been previously plugged with cotton and sterilized by dry heat at 150° C. for one hour. Care should be taken in filling the tubes to avoid the formation of air bubbles, as they leave a permanently uneven surface when the serum has been coagulated by heat. To prevent this the end of the pipette or funnel which contains the serum should be inserted well into the test-tube. About 3 c.c. are sufficient for each tube if the small size is employed; if not, 5 c.c. are required. The tubes, having been filled to the required height, are now to be coagulated and sterilized. They are placed slanted at the proper angle and then kept for two hours at a temperature just below 95° C. For this purpose a Koch serum coagulator or a double boiler serves best, though a steam sterilizer will suffice. If the latter is used a wire frame must be arranged to hold the tubes at the proper inclination, and the degree of heat must be carefully watched, as otherwise the temperature may go too high, and if the serum is actually boiled the culture medium will be spoiled. After sterilization by this process the tubes containing the sterile, solidified blood serum can be placed in covered tin boxes, or stopped with sterile paraffined corks and kept for months. The serum thus prepared is quite opaque and firm.

Swab for Inoculating Culture Tubes.—The swab we use to inoculate the serum is made as follows: A stiff, thin, iron rod, six inches in length, is roughened at one end by a few blows of a hammer, and about this end a little absorbent cotton is firmly wound. Each swab is then placed in a separate glass tube, and the mouths of the tubes are plugged with cotton. The tubes and rods are then sterilized by dry heat at about 150° C. for one hour, and stored for future use. These cotton swabs have proved much more serviceable for making inoculations than platinum-wire needles or wooden sticks, especially in young children and in laryngeal cases. It is easier to use the cotton swab in such cases, and it gathers up so much more material for the inoculation that it has seemed more reliable.

For convenience and safety in transportation "culture outfits" have been devised, which consist usually of a small wooden box containing a tube of blood serum, a tube holding a swab, and a record blank. These "culture outfits" may be carried or sent by messenger or express to any place desired.

Directions for Inoculating Culture Tubes with the Exudate.—The patient is placed in a good light, and, if a child, properly held. The swab is removed from its tube, and, while the tongue is depressed with a spoon, is passed into the pharynx (if possible, without touching the tongue or other parts of the mouth), and is rubbed gently but firmly against any visible membrane on the tonsils or in the pharynx, and

then, without being laid down, the swab is immediately inserted in the blood-serum tube, and the portion which has previously been in contact with the exudate is rubbed a number of times back and forth over the whole surface of the serum. This should be done thoroughly, but it is to be gently done, so as not to break the surface of the serum. The swab should then be placed in its tube, and both tubes, thin cotton plugs having been inserted, are reserved for examination or sent to the laboratory or collecting station (as in New York City). If sent to the health department laboratories for examination the blank forms of report which usually accompany each "outfit" should be filled out and forwarded with the tubes.

Where there is no visible membrane (it may be present in the nose or larynx) the swab should be thoroughly rubbed over the mucous membrane of the pharynx and tonsils, and in the nasal cavities, and a culture made from these. In very young children care should be taken not to use the swab when the throat contains food or vomited matter, as then the bacteriological examination is rendered more difficult. Under no conditions should any attempt be made to collect the material shortly after the application of strong disinfectants (especially solutions of corrosive sublimate) to the throat. Cultures from the nostrils are often more successful if the nostrils are first cleansed with a spray of sterile normal salt solution.

Examination of Cultures.—The culture tubes which have been inoculated, as described above, are kept in an incubator at 37° C. for twelve hours, and are then ready for examination. When great haste is required, even five hours will often suffice for a sufficient growth of bacteria for a skilled examiner to decide as to the presence or absence of the bacilli. On inspection it will be seen that the surface of the blood serum is dotted with numerous colonies, which are just visible. No diagnosis can be made from simple inspection; if, however, the serum is found to be liquefied or shows other evidences of contamination the examination will probably be unsatisfactory.

In order to make a microscopic preparation a clean platinum needle is inserted in the tube and quite a large number of colonies are swept with it from the surface of the culture medium, a part being selected where small colonies only are found. A sufficient amount of the bacteria adherent to the needle is washed off in the drop of water previously placed on the cover-glass and smeared over its surface. The bacteria on the glass are then allowed to dry in the air. The cover-glass is then passed quickly through the flame of a Bunsen burner or alcohol lamp, three times in the usual way, covered with a few drops of Loeffler's solution of alkaline methylene blue, and left without heating for five to ten minutes. It is then rinsed off in clear water, dried, and mounted in balsam. When other methods of staining are desired they are carried out in the proper way.

In the great majority of cases one of two pictures will be seen with the $\frac{1}{12}$ oil-immersion lens—either an enormous number of characteristic Loeffler bacilli, with a moderate number of cocci, or a pure cul-

ture of cocci, mostly in pairs or short chains. (See Streptococcus.) In a few cases there will be an approximately even mixture of Loeffler bacilli and cocci, and in others a great excess of cocci. Besides these, there will be occasionally met preparations in which, with the cocci, there are mingled bacilli more or less resembling the Loeffler bacilli. These bacilli, which are usually of the pseudodiphtheria type of bacilli (see Fig. 86), are especially frequent in cultures from the nose.

In not more than one case in twenty will there be any serious difficulty in making the diagnosis, if the serum in the tube was moist and had been properly inoculated. In such a case another culture must be made or the bacilli plated out and tested in pure culture.

Direct Microscopic Examination of the Exudate.—An immediate diagnosis without the use of cultures is often possible from a microscopic examination of the exudate. This is made by smearing a slide or cover-glass with a little of the exudate from the swab, drying, heating, staining, and examining it microscopically. This examination, however, is much more difficult, and the results are more uncertain than when the covers are prepared from cultures. The bacilli from the membrane are usually less typical in appearance than those found in cultures, and they are mixed with fibrin, pus, and epithelial cells. They may also be very few in number in the parts reached by the swab, or bacilli may be met with which closely resemble the Loeffler bacilli in appearance, but which differ greatly in growth and in other characteristics, and have absolutely no connection with them. When in a smear containing mostly cocci a few of these doubtful bacilli are present, it is impossible either to exclude or to make the diagnosis of diphtheria with certainty. Although in some cases this immediate examination may be of the greatest value, it is not a method suitable for general use, and should always be controlled by cultures.

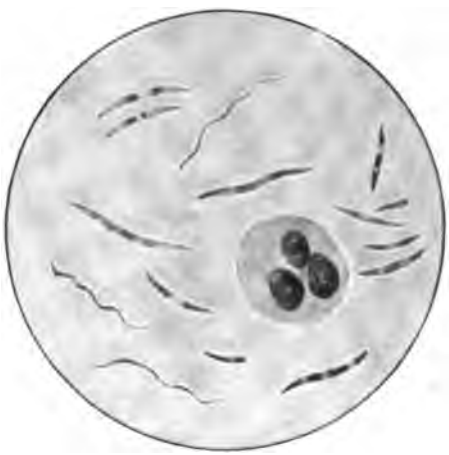
When carried out in the best manner an experienced bacteriologist may obtain remarkably accurate results. Higley in New York in a series of consecutive throat cases made the same diagnosis from the direct examination of smears as the Health Department laboratory made from the culture. To get the exudate he used a probe armed with a loop of heavy copper wire which has been so flattened as to act as a blunt curette. He makes thus thin smears from the exudate. After drying and fixing by heat the smears are stained for five seconds in a solution made by adding five drops of Kühne's carbolic methylene blue to 7 c.c. of tap water. After washing and drying stain for one minute in a solution of 10 drops of carbol-fuchsin in 7 c.c. of water. The dilute solution should be freshly prepared. The diphtheria bacilli will appear as dark-red or violet rods, and their contour, mode of division, and arrangement are manifest.

Animal Inoculation as a Test of Virulence.—If the determination of the virulence of the bacilli found is of importance, animal inoculations must be made. Experiments on animals form the only method of determining with certainty the virulence of the diphtheria bacillus. For this purpose, alkaline broth cultures of forty-eight hours' growth

should be used for the subcutaneous inoculation of guinea-pigs. The amount injected should not be more than one-fifth per cent. of the body-weight of the animal inoculated, unless controls with antitoxin are made. In the large majority of cases, when the bacilli are virulent, this amount causes death within seventy-two hours. If a good growth is not obtained in nutrient bouillon, ascitic broth should be used. At the autopsy the characteristic lesions already described are found. Bacilli which in cultures and in animal experiments have shown themselves to be characteristic may be regarded as true diphtheria bacilli, and as capable of producing diphtheria in man under favorable conditions.

For an absolute test of specific virulence antitoxin must be used. A guinea-pig is injected with antitoxin, and then this and a control animal, with 2 c.c. of a broth culture of the bacilli to be tested; if

FIG. 87



Vincent's Bacillus with accompanying Spirochætes.

the guinea-pig which received the antitoxin lives, while the control dies, it was surely a diphtheria bacillus which killed by means of diphtheria toxin—or, in other words, not simply a virulent bacillus, but a virulent diphtheria bacillus. When the bacilli to be tested grow poorly in a simple nutrient bouillon they should be grown in bouillon to which one-third its quantity of ascitic fluid has been added. Quite a number of bacilli have been met with which killed 250-grm. guinea-pigs in doses of 2 to 15 c.c., and yet were unaffected by antitoxin. These bacilli, though slightly virulent to guinea-pigs, produce no diphtheria toxin, and so cannot, to the best of our belief, produce diphtheria in man (see p. 206).

Vincent's Angina.—The local symptoms are similar to a slight case of diphtheria. Exudate or pseudomembrane forms on the tonsils and tends to become necrotic leaving a superficial ulcer, which is slow in healing. The general disturbance, outside a little fever, is

usually slight. The disease runs its course in from one to two weeks. It has been frequently noticed that the disease begins with an eruption of vesicles as in aphthous stomatitis. Paralysis never follows from this infection. The bacilli found by Vincent in the lesions are 6μ to 12μ long by 0.6μ to 0.8μ broad. Their ends are tapering. They are frequently bent like the letter S and resemble spirillæ.

The bacilli stain with methyl blue irregularly so that light and dark bands alternate (see Fig. 87).

Stained by the method of Romanowsky there appear sharply defined chromatin bodies in the blue stained protoplasm.

The bacilli are not motile.

These spindle-shaped bacilli have not been grown in pure culture, indeed there is doubt as to their nature, some considering them as being spirochætes. When direct smears are made from the exudate tiny spirochætes are usually found mixed with the bacilli.

Certain necrotic conditions of the mucous membrane of the cheek and about the teeth are accompanied by microörganisms very similar to those described by Vincent.

CHAPTER XVIII.

THE BACILLUS AND THE BACTERIOLOGY OF TETANUS.

TETANUS is a disease which is characterized by a gradual onset of general spasm of the voluntary muscles, commencing in man most often in those of the jaw and neck, and extending in severe cases to all the muscles of the body. The disease is usually associated with a wound received from four to fourteen days previously.

In 1884 Nicolaier, under Flügge's direction, produced tetanus in mice and rabbits by the subcutaneous inoculation of particles of garden earth. The Italians, Carle and Rattone, had just before demonstrated that the pus of an infected wound from a person attacked with tetanus could produce the same disease in rabbits, and showed that the disease was transmissible by inoculation from these animals to others. Finally, Kitasato, in 1889, obtained the bacillus of tetanus in pure culture and described his method of obtaining it and its biological characters.

Occurrence in Soil, etc.—The tetanus bacillus occurs in nature as a common inhabitant of the soil, at least in places where manure has been thrown, being abundant in many localities, not only in the superficial layers, but also at the depth of several feet. It has been found in many different substances and places—in hay-dust, in horse and cow manure (its normal habitat is the intestine of the herbivora), in the mortar of old masonry, in the dust from horses' hair; in the dust in rooms of houses, barracks, and hospitals; in the air, and in the arrow poison of certain savages in the New Hebrides, who obtained it by smearing the arrow-heads with dirt from crab holes in the swamps.

The tetanus bacilli are apparently more numerous in certain localities than in others—for example, some parts of Long Island and New Jersey have become notorious for the number of cases of tetanus caused by small wounds—but they are very generally distributed, as the experiments on animals inoculated with garden earth have shown, and are fairly common in New York City. In some islands and countries in the tropics cases of puerperal tetanus and tetanus in the newborn are very frequent. Tetanus bacilli are found in the intestines of about 15 per cent. of horses and calves living in the vicinity of New York City. They are also present to a somewhat less extent in the intestines of other animals and of man.

Morphology.—From young gelatin cultures the bacilli appear as motile, slender rods, with rounded ends, 0.5μ to 0.8μ in diameter by 2μ to 4μ in length, usually occurring singly, but, especially in old cultures, often growing in long threads. They form round spores, thicker than the cell (from 1μ to 1.5μ in diameter), occupying one of

its extremities and giving to the rods the appearance of small pins (Fig. 88).

Staining.—It is *stained* with the ordinary aniline dyes, and is not decolorized by Gram's method. The spores are readily stained and may be demonstrated by double-staining with Ziehl's method. The flagella are fairly easily stained in very young cultures.

Biology.—An *anaërobic, liquefying, moderately motile* bacillus. It has abundant peritrichic flagella. *Forms spores*, and in the spore stage it is not motile. It grows slowly at temperatures from 20° to 24° C., and best at 38° C., when, within twenty-four hours, it forms spores. It will not in pure culture grow in the presence of oxygen, but grows well in an atmosphere of hydrogen gas. With certain other bacteria the tetanus bacillus grows luxuriantly in the presence of oxygen.

Growth in Media.—The bacillus of tetanus grows in ordinary nutrient gelatin and agar of a slightly alkaline reaction. The addition to the media of 1.5 per cent. of glucose causes the development to be more rapid and abundant. It also grows abundantly in alkaline bouillon in an atmosphere of hydrogen. On *gelatin plates* the colonies develop slowly; they resemble somewhat the colonies of the *Bacillus subtilis*, and have a dense, opaque centre surrounded by fine, diverging rays. Liquefaction takes place more slowly, however, than with *Bacillus subtilis*, and the resemblance to these colonies is soon lost.

FIG. 88

Tetanus bacilli with spores in distended ends. × 1100 diameters.

The colonies on *agar* are quite characteristic. To the naked eye they present the appearance of light, fleecy clouds; under the microscope, a tangle of fine threads.

The *stab cultures in gelatin* exhibit the appearance of a cloudy, linear mass, with prolongations radiating into the gelatin from all sides (arborescent growth). Liquefaction takes place slowly, generally with the production of gas. In *stab cultures in agar* a growth occurs not unlike in structure that of a miniature pine-tree. *Alkaline bouillon* is rendered somewhat turbid by the growth of the tetanus bacillus. In all cases a production of gas results, accompanied by a characteristic and very disagreeable odor. It develops in *milk* without coagulating it.

Resistance of Spores to Deleterious Influences.—The spores of the tetanus bacillus are very resistant to outside influences; in a desiccated condition they may retain their vitality for several years, and are not destroyed in two and a half months when present in putrefying material. They withstand an exposure of one hour to 80° C., but are killed by an exposure of ten minutes at 105° C. to live steam. They resist the action of 5 per cent. carbolic acid for ten hours. A 5 per cent. solution of carbolic acid, however, to which 0.5 per cent. of hydrochloric acid has been added, destroys them in two hours. They are killed when acted upon for three hours by bichloride of mercury (1:1000), and in thirty minutes when 0.5 per cent. HCl is added to the solution. Silver nitrate solutions destroy the spores of

average resistance in one minute in 1 per cent. solution and in about five minutes in 1:1000 solution.

With regard to the persistence of tetanus spores upon objects where they have found a resting place, Henrijean reports that by means of a splinter of wood which had once caused tetanus he was able after eleven years again to cause the disease by inoculating an animal with the infective material.

Isolation of Pure Cultures.—The growth of the tetanus bacillus in the animal body is comparatively scanty, and is usually associated with that of other bacteria; hence, the organism is difficult to obtain in pure culture. The method of procedure proposed by Kitasato, which, however, is not always successful, consists in inoculating slightly alkaline nutrient agar or glucose bouillon with the tetanus-bearing material (pus or tissue from the inoculation wound), keeping the culture under anaërobic conditions for twenty-four to forty-eight hours at a temperature of 37° C., and, after the tetanus spores have formed, heating it for one-half an hour at 80° C., to destroy the associated bacteria. The spores of the tetanus bacillus are able to survive this exposure, so that when anaërobic cultures are then made in the usual way the tetanus colonies develop. When the tetanus bacilli are the only spore-bearing bacteria present, pure cultures are readily obtained; when other spore-bearing anaërobic bacteria are present, the isolation of a pure culture may be a matter of difficulty, but even then the presence of tetanus toxin in the culture fluid will indicate the presence of tetanus bacilli. The tetanus cultures can be kept for years.

Pathogenesis.—In mice, guinea-pigs, rabbits, horses, goats, and a number of other animals inoculations of pure cultures of the tetanus bacillus cause typical tetanus after an incubation of from one to three days. A mere trace of an old culture—only as much as remains clinging to a platinum needle—is often sufficient to kill very susceptible animals like mice and guinea-pigs. Other animals require a larger amount. Rats and birds are but little susceptible, and fowls scarcely at all. Man is more susceptible than any of the animals so far tested. A horse is about six times as sensitive as a guinea-pig and three hundred thousand times as sensitive as a hen. It is a remarkable fact that an amount of toxin sufficient to kill a hen would suffice to kill 500 horses. It is estimated that if 1 gram of horse requires 1 part of toxin to kill, then 1 gram of guinea-pig requires 6 parts, 1 of mouse 12, of goat 24, of dog 500, of rabbit 1500, of cat 6000, of hen 360,000. Cultures from different cases vary greatly in their toxicity. On the inoculation of less than a fatal dose in test animals a local tetanus may be produced, which lasts for days and weeks and then ends in recovery. On killing the animal there is found at autopsy, just at the point of inoculation, a hemorrhagic spot, and no changes other than these here or in the internal organs. A few tetanus bacilli may be detected locally with great difficulty, often none at all; possibly a few may be found in the region of the neigh-

boring lymphatic glands. From this scanty occurrence of bacilli the conclusion has been reached that the bacilli of tetanus, when inoculated in pure culture, do not multiply to any great extent in the living body, but only produce lesions through the absorption of the poison which they develop at the point of infection. It has been found that pure cultures of tetanus, after the germs have sporulated and the toxins been destroyed by heat, can be injected into animals without producing tetanus. But if a culture of non-pathogenic organisms is injected simultaneously with the spores, or if there is an effusion of blood at the point of injection, or if there was a previous bruising of the tissues, the animals surely die of tetanus. Even irritating foreign bodies have been introduced along with the spores deprived of their toxins, and tetanus did not develop; but if the wounds containing the foreign bodies became infected with other bacteria, tetanus developed and the animal died. From such experiments it seems that a mixed infection aids greatly in the development of tetanus when the infection is produced by spores not accompanied by tetanus toxin.

Natural Infection.—Here the infection may be considered as probably produced by the bacilli in their spore state, and the conditions favoring infection are almost always present. A wound of some kind has occurred, penetrating at least through the skin, though perhaps of a most trivial character, such as might be caused by a dirty splinter of wood, and the bacilli or their spores are thus introduced from the soil in which they are so widely distributed. If in any given case, the tissues being healthy, the ordinary saprophytic germs are killed by proper disinfection at once, a mixed infection does not take place, and tetanus will not develop. If, however, the tissues infected be badly bruised or lacerated, the spores may develop and produce the disease. Gelatin is occasionally found to contain tetanus spores.

Tetanus in Man.—Man and almost all domestic animals are subject to tetanus. It is a comparatively rare disease except after the Fourth of July celebration, when throughout the United States a considerable number of cases develop. In some years more than one hundred persons develop tetanus after blank cartridge wounds. On examination of an infected individual very little local evidence of the disease can be discovered. Generally at the point of infection, if there is an external wound, some pus is to be seen, in which, along with numerous other bacteria, tetanus bacilli or their spores may be found. Although rather deep wounds are usually the seat of infection, at times such superficial wounds as an acne pustule or a vaccination may give the occasion for infection. Not only undoubted traumatic tetanus, but also all the other forms of tetanus, are now conceded to be produced by the tetanus bacillus—puerperal tetanus, tetanus neonatorum, and idiopathic tetanus. In tetanus neonatorum infection is introduced through the navel, in puerperal tetanus through the inner surface of the uterus. It should be borne in mind that when there is no external and visible wound there may be an internal

one. The lesions in the nervous system are still obscure. Congestion, cellular exudate into the perivascular spaces, and chromatolysis of the ganglion cells are common. This is a pure toxæmic disease.

Toxins of the Tetanus Bacillus.—It is evident from the localization of the tetanus bacilli at the point of inoculation and their slight multiplication at this point that they exert their action through the production of powerful toxins. These toxins are named, according to their action, the tetanospasmin and the tetanolysin. One one-thousandths of a cubic centimeter of the filtrate of an eight-day glucose bouillon culture of a fully virulent bacillus is sufficient to kill a mouse. The purified and dried tetanus toxin prepared by Brieger and Cohn was surely fatal to a 15-gram mouse in a dose of 0.000005 gram. The appalling strength of tetanus toxin may readily be appreciated when it is stated that it is twenty times as poisonous as dried cobra venom.

The quantity of the toxin produced in nutrient media varies according to the age of the culture, the composition of the culture fluid, reaction, completeness of the exclusion of oxygen, etc. For some reason more toxin develops in broth inoculated with masses of tetanus spores than with bacilli. The variation in strength is partly due to the extreme sensitiveness of the toxin, which deteriorates on keeping or on exposure to light, being also sensibly affected by most chemical reagents and destroyed by heating to 55° to 60° C. for any length of time. It retains its strength best when protected from heat, light, oxygen, and moisture. Under the best conditions the amount of toxin produced in cultures by the fifth day is such that 0.000005 c.c. is the fatal dose for a 15-gram mouse.

The tetanus cultures retain their ability to produce toxins unaltered when kept under suitable conditions; but when subjected to deleterious influences they may entirely lose it. The usual medium for the development of the toxin is a slightly alkaline bouillon containing 1 per cent. of peptone and 0.5 per cent. salt. In addition 1 per cent. of dextrose is sometimes added but is not advised.

Action of Tetanus Toxin in the Body.—After the absorption of the poison there is a lapse of time before any effects are noticed. With an enormous amount, such as 30,000 fatal doses, this is about twelve hours; with ten fatal doses, thirty-six to forty-eight hours; with two fatal doses, two to three days. Less than a fatal dose will produce local symptoms. The parts first to be affected with tetanus are, in about one-third of the cases in man, and usually in animals, the muscles lying in the vicinity of the inoculation—for instance, the hind foot of a mouse inoculated on that leg is first affected, then the tail, the other foot, the back and chest muscles on both sides, and the forelegs, until finally there is a general tetanus of the entire body. In mild cases, or when a dose too small to be fatal has been received, the tetanic spasm may remain confined to the muscles adjacent to the point of inoculation or infection. The symptoms following a fatal dose of toxin vary greatly with the method of injection. Intraperitoneal injection is

followed by symptoms which can hardly be distinguished from those due to many other poisons. Injection into the brain is followed by restlessness and epileptiform convulsions. The tetanus toxins undoubtedly combine readily with the cells of the central nervous system. They also combine with other tissue cells with less apparent effects. The symptoms in tetanus depend upon an increased reflex excitability of the motor cells of the spinal cord, the medulla, and pons.

Presence of Tetanus Toxin in the Blood of Infected Animals.—The blood usually contains the poison, as has been proved experimentally on animals. Neisser showed that the blood of a tetanic patient was capable of inducing tetanus in animals when injected subcutaneously. In St. Louis the serum of a horse dying of tetanus was given by accident in doses of 5 to 10 c.c. to a number of children, with the development of fatal tetanus. In this connection Bolton and Fisch showed by a series of experiments that much toxin might accumulate in the serum before symptoms became marked. Ehrlich has shown that besides the predominant poison which gives rise to spasm (tetanospasmin) there exists a poison capable of producing solution of red blood corpuscles. This he calls tetanolysin. It was not found in all culture fluids. Whether in actual disease this poison is ever in sufficient amount to cause appreciable harm is not known. After one or two weeks the blood becomes antitoxic even though the symptoms persist.

Tetanus Antitoxin.—Behring and Kitasato were the first to show the possibility of immunizing animals against tetanus infection. The treatment of tetanus is directed against the action of the toxin and this is accomplished by the neutralization of the toxin by antitoxin in the body.

The immunizing experiments in tetanus have borne practical fruit, for it was through them that the principle of serum therapeutics first became known—the protective and curative effects of the blood serum of immunized animals. It was found that animals could be protected from tetanus infection by the previous or simultaneous injection of tetanus antitoxin, provided that such antitoxic serum was obtained from a thoroughly immunized animal. From this it was assumed that the same result could be produced in natural tetanus in man. Unfortunately, however, the conditions in the natural disease are very much less favorable, inasmuch as treatment is usually commenced not shortly after the infection has taken place, but only many hours after the appearance of tetanic symptoms, when the poison has already attacked the cells of the central nervous system.

The tetanus antitoxin is developed in the same manner as the diphtheria antitoxin—by inoculating the tetanus toxin in increasing doses into horses. The toxin is produced in bouillon cultures grown anaërobically for six to ten days. Abundant spores should be used to inoculate the broth. The culture fluid is filtered through porcelain, and the germ-free filtrate is used for the inoculations. The horses receive 5 c.c. as the initial dose of a toxin of which 1 c.c. kills 250,000

grams of guinea-pig, and along with this twice the amount of antitoxin required to neutralize it. In five days this dose is doubled, and then every five to seven days larger amounts are given. After the third injection the antitoxin is omitted. The dose is increased at first slowly until appreciable amounts of antitoxin are found to be present and then as rapidly as the horses can stand it, until they support 700 to 800 c.c. or more at a time. This amount should not be injected in a single place, or severe local and perhaps fatal local tetanus may develop. After some months of this treatment the blood of the horse contains the antitoxin in sufficient amount for therapeutic use. Some horses have produced as high as 600 units per c.c.

Antitoxin Unit and Technique of Testing Antitoxin Serum.—Tetanus antitoxin is tested exactly in the same manner as diphtheria antitoxin, except that the unit is different. In April, 1907, the producers of serum in the United States agreed to a unit of antitoxin which is approximately ten times the size of the unit of diphtheria antitoxin. A unit is defined as the amount of antitoxin required to just neutralize 1000 fatal doses of tetanus toxin for a 350-gram guinea-pig. If the test guinea-pig, receiving the mixture of antitoxin, and 1000 times the amount, is protected from death for four days, neutralization is considered to have taken place. The United States government has adopted this unit and supplies the different producers with standardized toxin.

The amount of antitoxic serum which neutralizes an amount of test toxin which would destroy 40,000,000 grams of mouse contains 1 unit of antitoxin by the German standard. In the French method the amount of antitoxin which is required to protect a mouse from a dose of toxin sufficient to kill in four days is determined, and the strength of the antitoxin is stated by determining the amount of serum required to protect 1 gram of animal. If 0.001 c.c. protected a 10-gram mouse the strength of that serum would be 1 : 10,000. The toxin used for testing is preserved by precipitating it with saturated ammonium sulphate and drying and preserving the precipitate in sealed tubes. As required, it is dissolved in 10 per cent. salt solution as above stated. For small testing stations the best way is to obtain some freshly standardized antitoxin and compare serums with this.

Persistence of Antitoxin in the Blood.—Ransom has clearly shown that the tetanus antitoxin, whether directly injected or whether produced in the body, is eliminated equally rapidly from the blood of an animal, provided that the serum was from an animal of the same species. If from a different species it is much more quickly eliminated. From this we see a probable explanation of the fact that immunity in man, due to an injection of the antitoxic serum of the horse, is less persistent than immunity conferred by an attack of the disease.

The same author found some interesting facts in testing the antitoxic values of the serum of an immunized mare, of its foal, and of the milk. The foal's serum was one-third the strength of the mare's and one hundred and fifty times that of the mare's milk. In two months the mare's serum lost two-thirds in antitoxic strength, the

foal's five-sixth, and the milk one-half. Injections of toxin were then given the mare, so that it doubled its original strength in one month. The milk increased eightfold, but the foal's continued to lose in antitoxin, although it was feeding on antitoxic milk. Under diphtheria it was noted that homologous antitoxin remained much longer than heterologous.

Toxin and Antitoxin in the Living Organism. Animal Experiments.—The experiments of Meyer and Ransom and of Marie and Morax have proved to them that the poison is transported to the central nervous system by the way of the motor nerves—and by no other channel. These authors thought that they had shown that the essential element for the absorption and transportation of the toxin is not the nerve sheath or the lymph channels, but the axis-cylinder, the intramuscular endings of which the toxin penetrates. The poison is taken up quite rapidly. Marie and Morax were able to demonstrate the poison in the corresponding nerve trunk (sciatic) one and a half hours after the injection. Absorption, however, and conduction are dependent to a large extent on the nerves being intact. A nerve cut across takes very much longer to take up the poison (about twenty-four hours), and a degenerated nerve takes up no poison whatever. In other words, we see that section of the nerve prevents the absorption of the poison by way of the nerve channels. Similarly section of the spinal cord prevents the poison from ascending to the brain.

According to Meyer and Ransom, the reason why the sensory nerves do not play any rôle in the conduction of the poison lies in the presence of the spinal ganglion, which places a bar to the advance of the poison. Injections of toxin into the posterior root leads to a tetanus dolorosus, which is characterized by strictly localized sensitiveness to pain.

Ascending centripetally along the motor paths, the poison reaches the motor spinal ganglia on the side of inoculation; then it affects the ganglia of the opposite side, making them hypersensitive. The visible result of this is the highly increased muscle tonus—*i. e.*, rigidity. If the supply continues, the toxin next affects the nearest sensory apparatus; there is an increase in the reflexes, but only when the affected portion is irritated. In the further course of the poisoning the toxin as it ascends continues to affect more and more motor centres, and also the neighboring sensory apparatus. This leads to spasm of all the striated muscles and general reflex tetanus.

A different explanation of the passage of the toxin up the nerve trunks has recently been discovered. It is well known that the lymph flow in nerves is from the periphery to the center, and Field in our laboratory has shown that not only tetanus toxin, but diphtheria toxin and inert colloids can be demonstrated in the sciatic nerves after they have been injected subcutaneously or intramuscularly, and after varying periods may be found in the spinal cord. He believes that the toxins are absorbed by way of the lymphatics of the nerves, and not by way of the axis-cylinder.

A recent experiment of Cernovodeanu and Henni almost proves this contention. They ligated all the muscles and blood vessels in a guinea-pig's leg, leaving intact only the sciatic nerve, skin and bone, and then injected a large amount of tetanus toxin below the point of ligation. The animals in which this was done never developed tetanus.

In this case there was only a very slight flow of lymph into the ligated area, and so there could be only a slight flow up the nerve.

If the toxin gets into the blood the only path of absorption to the central nervous system is still by way of the motor-nerve tracts. There seems to be no other direct path, as, for example, by means of the blood vessels supplying the central nervous system. Even after introducing the poison into the subarachnoid space, owing to the passage of the poison into the blood, there is a general poisoning and not a cerebral tetanus. This at least is the case if care has been taken during the operation to avoid injuring the brain mechanically.

Rapidity of Absorption of Tetanus Antitoxin from Tissues.— The complete absorption of a given quantity of antitoxin administered subcutaneously takes place rather slowly. In his animal experiments Knorr found the maximum quantity in the blood only after twenty-four hours. From that time on the amount again steadily decreased, so that by the sixth day only one-third the optimum quantity was present. By the twelfth day only one-fiftieth and at the end of three weeks no antitoxin whatever could be demonstrated. These facts emphasize the necessity of giving the first dose in a case of tetanus intravenously.

Naturally the time during which these changes take place varies with the application, the conditions of absorption, and the concentration and amount of the preparation injected. When injected intravenously the antitoxin very quickly passes into the lymph. Ransom was able to demonstrate it in the thoracic duct of a dog a few minutes after intravenous injection. *Neither the central nervous system nor the peripheral nervous tissue take up any antitoxin from the blood.* Only after very massive intravenous doses are small traces found in the cerebrospinal fluid. From this it is at once clear that passively and actively immunized animals become tetanic if the poison is injected directly into the central nervous system or into a peripheral nerve. Antitoxin injected subdurally also passes almost entirely over into the blood.

A rapid and plentiful appearance of antitoxin in the blood is dependent on the content of serum in antitoxin units. The more units, the more rapidly will the blood develop a high content of antitoxin; and the higher this is the more thoroughly will the tissue fluids be saturated with the antitoxin.

From the foregoing it is not difficult to formulate the conditions under which an antitoxin introduced into the organism can exert its neutralizing power on the toxin. We see that the poison deposited at any given place takes either of two paths to the central nervous

system, one a direct path by way of the local peripheral nerves and the other an indirect path through the lymph channels and blood to the end plates of all other motor nerves. Only that portion can be neutralized which (*a*) still lies unabsorbed at the site of inoculation, or (*b*) which, though it has passed into the blood, has not yet been taken up by the motor-nerve endings. A curative effect can therefore result from antitoxin introduced subcutaneously or intravenously only so long as a fatal dose of poison has not been taken up by the nerves.

So long as the toxin circulates in the blood it is neutralized by antitoxin in about the same proportion as in test-tube experiments. By means of intravenous injections of antitoxin Ransom was able to render the blood free from toxin in a very few minutes. According to Marie and Morax, toxin injected into the muscles is already demonstrable in the nerve tissue at the end of one and a half hours—*i. e.*, it has already entered the channel, where it is no longer reached by the antitoxin. Dönitz injected various rabbits intravenously each with 1 c.c. of a toxin solution containing twelve fatal doses. Thereupon he determined the dose of antitoxin which, when intravenously given, would neutralize this poison after various intervals of time. The antitoxin was of such a strength that in test-tube experiments 1 c.c. of a 1 : 2000 solution just neutralized the amount of toxin employed. He found that at the end of two minutes double the dose required *in vitro* would still neutralize the poison; at the end of four minutes about four times the dose was required, and at the end of eight minutes ten times. When one hour had been allowed to elapse forty times the original dose just sufficed to protect the animal from death, but not from sickness. In order to explain these results, the correctness of which has been confirmed by many analogous observations, the conception "loose union of toxin" has been introduced. By this is meant a state of union between toxin and susceptible cell constituent which can still be disrupted by means of large doses of antitoxin. In this particular instance we do not need to make use of this conception, for the reason that the tetanus toxin is not at all combined during the first hour. Personally, we should regard it as more probable that the interval during which the toxin can still be neutralized, though with difficulty, corresponds to that time during the passage of the toxin in which after leaving the capillaries the poison is held up in the fine interstices of the connective tissue which it must penetrate before it can be taken up by the nerves.

Results of the Antitoxin Treatment in Tetanus.—The course of tetanus varies so much with the individual that it is difficult to judge by statistics or personal experience as to the value of the antitoxic treatment of the developed form. It is interesting to note that the two latest authoritative reviews by American writers differ greatly in their conclusions. McFarland, who has had an extensive experience, states, "It would seem, therefore, that we have in tetanus antitoxin not a specific, because it fails too often to have merited that

name, but a valuable remedy in the treatment of the disease, and one that ought not to be neglected until a better one is supplied." Our own opinion, founded on reading and a considerable personal experience is even more favorable. We have seen numerous cases of generalized tetanus that after a large intravenous injection have markedly improved and finally recovered, and these cases have certainly done better on the average than apparently similar ones receiving palliative treatment alone. Lambert, who some years ago made an exhaustive study of tetanus, states that in a total of 114 cases of this disease treated with antitoxin, according to published and unpublished reports, there was a mortality of 40.35 per cent. Of these, 47 were acute cases—that is, cases with an incubation period of eight days or less and with rapid onset, or cases with a longer period of incubation, but intensely rapid onset of symptoms; of these the mortality was 74.46 per cent. Of the chronic type—those with an incubation period of nine days or more, or those with shorter incubation with slow onset—there were 61 cases, with a mortality of 16.39 per cent. With a still larger number of cases the results indicate that with tetanus antitoxin about 20 per cent. better results are obtained than without. In our own diphtheria antitoxin horses we used to lose several almost every year from tetanus infection until we immunized all the animals every three months with about 5000 units of tetanus antitoxin.

Methods of Administering Tetanus Antitoxin.—For immunization, about 1500 units of a serum of medium strength will suffice, unless the danger seems great, when the injection is repeated at the end of a week. For treatment, begin with an intravenous injection of 10,000 units, and repeat every eight to twelve hours until the symptoms abate. It is well to continue decreasing daily injections until recovery is certain. In some of the gravest cases no curative effect will be noticed from the serum. The first injections should be made intravenously, or partly intravenously and partly into the spinal canal through lumbar puncture. Later, injections should be made subcutaneously or intravenously. Besides these, injections are advised by some to be made into all the nerve trunks leading from the infected region. These injections are directed to be made as near the trunk as possible and distend the nerve so as partly to neutralize and partly mechanically interrupt the passage of toxin to the cord or brain. In New York City Rogers believes he has had good results by following these methods. The method of injecting from 3 to 15 c.c. of antitoxic serum into the lateral ventricles has not, in the writer's opinion, shown itself to be advisable. No bad results have followed the injections when the serum was sterile and the operation was performed aseptically; but several brain abscesses have already followed the intracerebral injections.

The striking results which have been obtained, both in human and in veterinary practice, with the prophylactic injection of tetanus antitoxin, would seem to warrant the treating of patients with immunizing doses of serum—at least, in neighborhoods where tetanus is not

uncommon—when the lacerated and dirty condition of their wounds may indicate the possibility of a tetanus infection.

Splendid results have followed this practice in many places. It is the custom at many dispensaries in New York City and elsewhere to immunize all Fourth of July wounds by injecting 1000 units. None of these have ever developed tetanus. Even the eleven cases of human tetanus reported as occurring in Europe after single injections of antitoxin prove the value of immunizing injections, for the mortality was only 27 per cent. They teach also that where tetanus infection is suspected the antitoxic serum should be given a second and even a third time at intervals of seven days.

In coöperation with Dr. Cyrus W. Field, we have recently tried a number of experiments upon guinea-pigs to test the importance of intravenous and of intraneural injections of antitoxin in animals in which tetanus had already developed. Forty guinea-pigs have been experimented upon. These were injected in the lower part of the hind leg with ten to twenty times the fatal dose of a mixture of tetanus toxin and bacilli. Within from one to two hours after the development of the first definite symptoms of tetanus the animals were operated upon and given antitoxin. The experiments show clearly that moderate doses of antitoxin given after the development of tetanus did not save the animals from death or even prolong life, while very large doses usually did both. Seventy-five per cent. of those receiving 500 units recovered. The surprising result developed that amputation of the infected leg at the hip-joint hastened the death of the animals in every case. • Control animals which had not been infected stood the amputation perfectly well, and made good recoveries. Without antitoxin, excision of a piece of the nerve did not materially prolong life, nor did ligation of the nerve. In the guinea-pigs receiving antitoxin the ligation of the nerve seemed to be of benefit. The results of the experiments showed that large doses of antitoxin given shortly after the development of tetanus usually saved the animals, and that most of the toxin was absorbed by the blood and not by the nerves of the infected part. Every minute of delay after the appearance of tetanus was of importance. We feel convinced that in human tetanus the most important thing is to give at the earliest possible moment after diagnosis a very large intravenous injection of antitoxin. From 50 to 75 c.c. of the most potent serum obtainable should be given. During succeeding days injections can be given either intravenously or subcutaneously until marked improvement or death has taken place. If a surgeon is at hand intraneural injections into the nerves supplying the infected portion of the body may also be given, but these, we believe, are not usually necessary if the large intravenous injections have been given.

Differential Diagnosis.—The differential diagnosis of the bacillus of tetanus is, generally speaking, not difficult, inasmuch as animal inoculation affords a sure test of the specific organism. No other microörganism known produces similar effects to the tetanus bacillus, nor is any other neutralized by tetanus antitoxin. The other charac-

teristics also of this bacillus are usually distinctive, though microscopic examination alone cannot be depended on to make a differential diagnosis. Difficulty arises when other anaërobic or aërobic bacilli, almost morphologically identical with the tetanus bacillus, are encountered which are non-pathogenic, such as the *Bacillus pseudotetanicus anaërobius*, already mentioned, and the *Bacillus pseudotetanicus aërobius*. It is possible, however, that both these bacilli, when characteristic in cultures, are only varieties of the tetanus bacillus, which, under unfavorable conditions of growth, have lost their virulence. These non-virulent types do not, as a rule, have spores absolutely at their ends, and the spores themselves are usually more ovoid than those in the true tetanus bacilli.

Methods of Examination in a Case of Tetanus.—(*a*) *Microscopic.*—From every wound or point of suppuration film preparations should be made and stained with the usual dyes. The typical spore-bearing forms are looked for, but are usually not found. At the same time other bacteria are noted if present.

(*b*) *Cultures.*—Bits of tissue, pus, cartridge wads, etc., are collected and dropped into glucose bouillon contained in small flasks or tubes. This bouillon should be slightly alkaline, be free from oxygen, and protected from oxygen. A simple way is to cover the bouillon with liquid or semi-solid paraffin, and thus boil and afterward cool it. Cultures placed in such protected bouillon grow readily.

(*c*) *Inoculation.*—A salt solution emulsion of material from the wound is inoculated into mice or guinea-pigs subcutaneously.

CHAPTER XIX.

INTESTINAL BACTERIA.

Significance of Bacteria in Intestines.—The constant presence of great numbers of bacteria in the intestinal tract has been the subject of much investigation which has given somewhat conflicting results. On the one hand, certain experiments seem to show that the presence of intestinal bacteria is not essential to life. For example, Nuttall and Thierfelder experimenting with guinea-pigs succeeded in keeping the intestines free from bacteria for a limited time, during which the young pigs remained well. Furthermore, Levin makes the interesting statement that the intestinal tract of polar animals is for the most part sterile.

On the other hand, the supporters of the opposite theory, namely, that certain intestinal bacteria are necessary for perfect physiologic action, state that in their experiments on feeding animals with sterile food they found that development was retarded; thus Schottelius claims this for chickens, and Mme. Metchnikoff obtained similar results with young frogs. However, whether or not the presence of bacteria in the intestinal canal is essential to the animal economy, it is, nevertheless, evident that microörganisms play a certain rôle in aiding or inhibiting some of the alimentary processes dependent upon biological activity. Recently new interest has been added to the subject by the work of Metchnikoff who claims that old age is hastened by the increased growth and action of certain putrefactive bacteria normally found in small numbers in the intestines; and he, Herter, and others consider that the development of these harmful varieties may be checked by the growth of the obligate intestinal bacteria or by some substituted variety which has no harmful action upon the host.

Conditions Influencing Development of Bacteria.—The intestinal canal presents such varying conditions dependent upon so many different factors that of necessity its flora will reflect great diversity. As the organisms gain access to the tract chiefly through the air, food, and drink ingested, the character of these will influence the nature of the flora. The condition of the oral cavity and that of the respiratory passages on account of swallowing bacteria will also have an influence on the kind of bacteria found. Some few microörganisms, such as the colon group and the obligate anaërobes have become established as regular inhabitants of the intestines and find in the different localities of the canal their best environment. Together with these may be found those bacteria which having been ingested with various substances have survived the action of the gastric and intestinal fluids.

The length of time which the intestinal contents are retained at any one point of the tract will cause an increase or decrease of certain types, as well as the total number, since all portions of the canal are not equally adapted to the development of any one species nor to bacteria as a class.

Under absolutely normal conditions organisms, which are not destroyed, pass through the intestinal tract without entering the body of the host, but if injury occurs to the intestinal wall or the normal resistance of the body's tissues is lowered for any reason they may pass into the circulation. Maklęzow claims that after twenty-two hours of fecal impaction, intestinal organisms were found in the circulation. MacFayden has also demonstrated the same. In chronic constipation intestinal bacteria are found in the urine.

Escherich found that *in utero* and immediately after birth the meconium is sterile, unless, in exceptional cases where the mother has suffered from a severe bacterial infection and the invading organisms are found in the fœtus. From three to seven hours after birth a few bacilli, cocci, and yeasts may be found, having presumably entered by the anus; after eighteen hours the number and kinds of bacteria increase, being taken in by the food or the swallowing of saliva. The stools of artificially-fed infants show a greater variety of organisms than those of the breast-fed child.

Anaërobic Conditions in the Intestines.—Virchow first questioned the presence of free oxygen in the entire intestinal canal and concluded it was essentially anaërobic, the oxygen which is taken in being quickly absorbed or combined with hydrogen.

The character of the flora also indicates an anaërobic condition of the small intestines with more or less aërobic conditions in the lower part of the colon and rectum.

The anaërobes play the chief part in intestinal putrefaction, and certain varieties are thought to be at least the predisposing if not the chief cause of many cases of appendicitis.

Regional Distribution of Bacteria in Digestive Tract.—Many different organisms may be found at times in all parts of the tract, but each species finds its best environment in some one location and is here found with greater frequency. In the stomach, very few bacteria develop, the sarcinæ, *B. gastricus*, and cloacæ group are rather constant, the larger number and variety taken in being destroyed to a great extent by the gastric juice.

The fact that great numbers of bacteria are destroyed by the digestive juices, together with a rapid passage of the partly digested food and the strict anaërobic condition, accounts for the very few bacteria that are usually found in the upper part of the small intestines. It is in this location that the obligate anaërobes, which are usually spore bearers and often Gram-positive organisms, such as the putrificus of Bienstock, capsulatus aërogenes and *B. bifidus* are usually found. The chief bacteria of the lower part of the small and the upper part of the large intestines are members of the *B. coli* group which reach their

highest development in the cæcum and upper colon. Here, too, other organisms which have been held in check by the above chemical and mechanical causes, finding a more suitable soil, develop, and a marked increase is found in many Gram-positive bacilli and cocci of various types. In the lower colon and rectum with the accumulation of specific antagonistic substances formed from the abundant growth higher up, many forms, especially of *B. coli* group, are more or less destroyed.

The flora can be materially changed in dogs by the diet, as has been shown by Herter. Lemke and MacFayden demonstrated the same in man.

The range of variation of the bacteria that appear normally from time to time in the intestinal tract is so very great that no one grouping, except in the most general sense, such as fermenters or non-fermenters of glucose, anaërobes, or aërobes, seems to apply to all cases. Ford isolated 50 distinct species from the human fæces.

Methods Used in Examination of Normal Fæces.—The material should be taken from a perfectly fresh stool, preferably after a dose of castor oil has been given. This induces a quick and thorough emptying of the intestinal tract, with the least alteration of the chemical nature of the fæces. The use of blind tubes or flushing are apt to give only the contents of the lower part of the colon and rectum and are useful only when the examination is to be limited to this area.

To 1 gram of the material 100 c.c. of normal salt solution is gradually added, first rubbing up the fæces in a small quantity of the diluent and then shaking thoroughly as more is added. Definite amounts of this emulsion can be used for plating. Two per cent. glucose agar with defibrinated blood added to it is very satisfactory for the isolation of anaërobes, while beerwort agar (10 per cent. sterile beerwort added to the usual stock agar) is used for the acidophile group. A very convenient method, according to Zinsser, of growing anaërobic cultures is to take crystallizing dishes of different sizes so that one dish fits within the other, leaving a space of three-fourths of an inch all around. The larger dish is placed over the smaller dish as a cover, they are then wrapped in filter-paper and in this way can be easily sterilized. When ready for plating 1 c.c. of defibrinated blood is placed in the bottom of the smaller dish and into this is poured 10 c.c. of glucose agar which has been inoculated with 1–10 c.c. of the above emulsion. By gently tipping the dish back and forth the blood and agar become very well mixed. This is covered with a petri dish or the companion crystallizing dish and allowed to stand until it is perfectly cold. In the larger dish are placed two pieces, about 1½ inches to 2 inches long, of caustic soda and the dish is filled to about ¼ its capacity with pyrogallic acid. The smaller dish is carefully inverted over this and sufficient sterile water poured into the larger dish to cover the acid. The whole is then sealed with paraffin or oil poured over the water that is collected outside the smaller dish; this prevents continual absorption of the oxygen from the air. Plates are made from beerwort agar and grown both anaërobically and aërobically. Fishings are made from these plates upon corresponding tube media and the cultures are further tested on such media as may be best adapted to each special organism.

Substitution of one Variety of Bacteria for Others.—It is possible for the usual flora of the intestines to be almost entirely replaced temporarily by an invading organism. This occurs in disease when the microörganism producing a specific disease of the intestines is

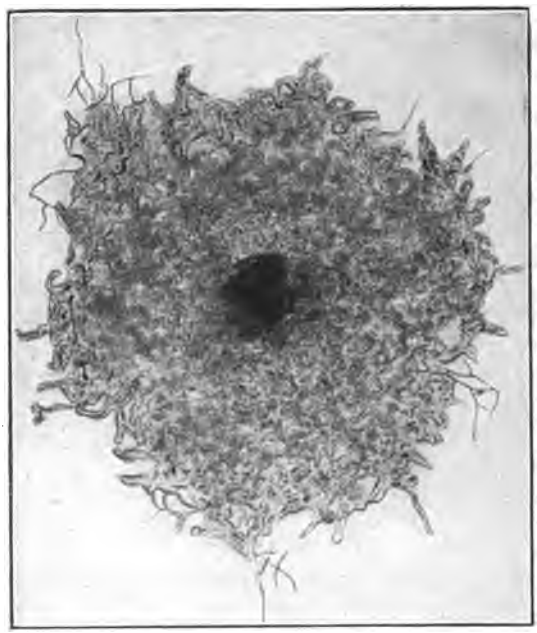
found in almost pure cultures, as in dysentery or cholera, or where a large number of organisms are swallowed, as in the case of a child that had dysentery, and the dysentery bacillus was found to be abundant; but few days after the onset of the attack, tonsillitis developed and the stools contained streptococci, greatly in excess of the other organisms.

As already stated, Metchnikoff claims that this possibility of substitution can be used in cases where intestinal putrefaction is excessive and thus check the process by the introduction of a lactic acid bacterium.

This work of Metchnikoff and his followers has led to an extensive study of organisms causing lactic acid fermentation. Our present knowledge of the lactic acid milks is summed up in the following paragraphs:

Lactic Acid Milks.—For many years the people of western Asia and eastern Europe have looked upon sour milk as an essential part of daily

FIG. 89



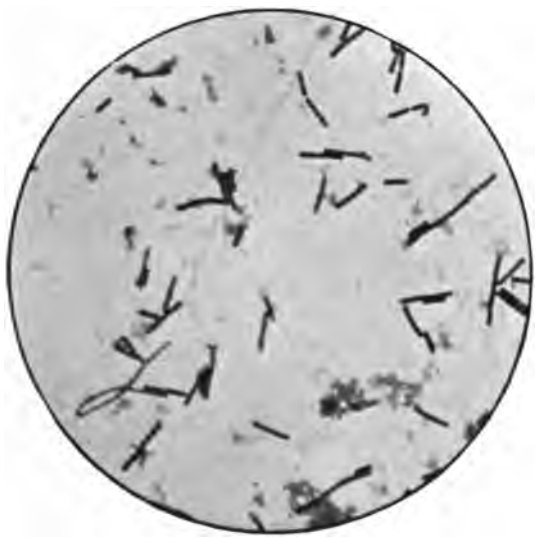
B. bulgaricus; 7th day (44°) colony. Whey agar plate. × 50 diameters. (White and Avery.)

diet. In western Europe and America buttermilk has been a favorite drink with many, but it never assumed as much importance as in the east. The term sour milk covers all milks or parts of milks in which lactic acid fermentation is pronounced. The ordinary buttermilk sours because of the growth of lactic acid bacteria in the raw milk which have been derived from the local surroundings. Sour milk

from the dealers may be such milk, but it is more usually heated milk to which some special culture of bacteria (starter) has been added. Sour milk is usually nearly fat-free, but more or less of the cream may remain in it. Hansen, of Copenhagen, has for some time supplied a lactic acid bacillus which has been much used. Another starter now popular is one supplied by Metchnikoff which he obtained from the east. There are a number of preparations of sour milk used at present, among these are:

Kumyss, in which the fermentation is due to lactic acid bacteria and yeasts, and thus contains not only lactic acid, but carbon dioxide and about 1 per cent. of alcohol.

FIG. 90



B. bulgaricus. × 1000 diams. (Piffard.)

Maadzoun and Yoghurt, the common sour milk of southeastern Europe, containing chiefly the *B. bulgaricus* and streptococci and diplococci, all producing lactic acid.

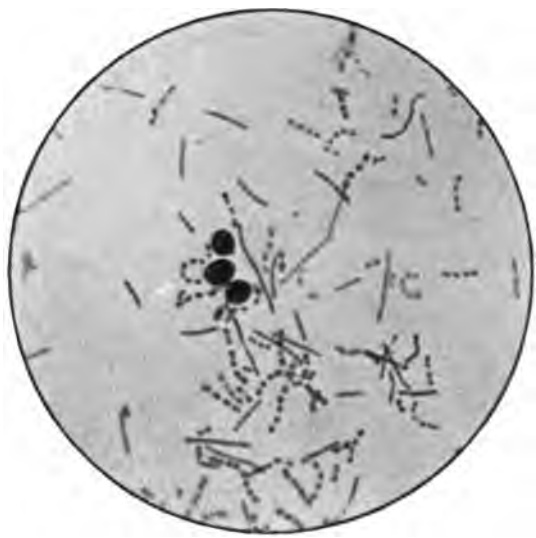
Zoolak (matzoon) made by adding to heated milk the same bacteria as occur in maadzoun.

Yohourd, blabberade, and other sour milks are made by the use of much the same organisms.

The bacilli at present of most interest are those resembling the *B. bulgaricus* (B. of Massol) which are present in the eastern milks and are now through the advocacy of Metchnikoff used alone or in connection with a lactic acid streptococcus to produce much of the souring of milk of Europe and America. In 1906, Cohendy studied the action of this bacillus and found that it produced a large amount of lactic acid, 3.23 per cent. being found after 10 days at 36° C. From other preparations slightly different bacilli were found which produce a firm clot, while the *B. bulgaricus* produces a soft curd. Some

bacilli which resemble the *B. bulgaricus* in many respects produce gas as well as acid. The bacilli in all strains of *B. bulgaricus* show wide variations in length from 2μ to 50μ. Chains of bacilli occur in some

FIG. 91



"Lactic acid" milk containing B. bulgaricus and a lactose fermenting streptococcus.

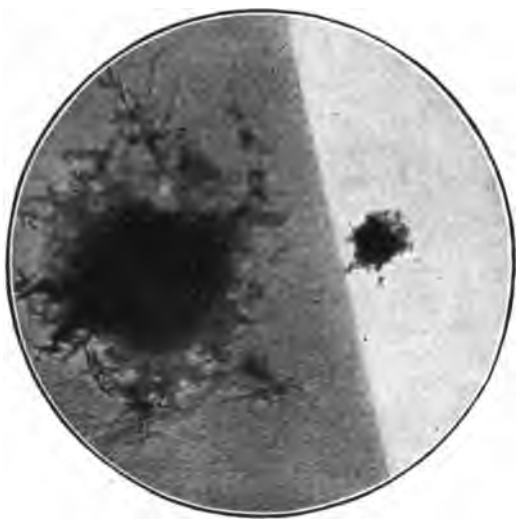
FIG. 92

Yeast cells and lactose fermenting bacilli in "fermented milk". × 1000 diams.

strains to a more marked degree than in others. The bacilli are non-motile, non-sporulating, Gram-positive, except when in involution forms, when they are said to be Gram-negative. Difficult to cultivate

in most media. When freshly isolated, growth obtained only on media containing whey or malt or milk. Grow equally well in aërobic and anaërobic conditions. Optimum temperature for growth is 44°, fair growth at 30°, slight at 25°, none at 20°. Gelatin is not liquefied. Colonies on whey agar are round, grayish-white, and measure 0.5 to 1.5 mm. Periphery of colonies mostly filamentous. The growth in whey produces clouding, but this disappears in 5 to 14 days, leaving a sediment. Coagulates milk in 8 to 18 hours at 44°, and after longer time at lower temperatures. The lactic acid formed is either inactive or lævorotatory. A small quantity of volatile acid is also produced. No appreciable peptonization of the curd.

FIG. 93



B. bulgaricus from normal mouth; 48-hour colonies on acid whey agar at 42° C. Small colony ×40 diameters. Large colony ×220 diameters (Heinemann and Hofferan).

The bacilli are non-pathogenic. These bacilli are probably widely distributed in nature, being frequently present in the intestines of man and animals. White and Avery, who have made an exhaustive study of this group of bacilli, consider that they all belong to one group which is identical with the group Bacterium caucasicum (Kern). There are apparently at least two distinct types which differ in the amount and kind of lactic acid formed.[1]

Prevalent Intestinal Bacteria.—B. Bifidus.—Tissier found the *B. bifidus* in the stools of breast-fed infants which at times forms nearly the entire flora. He found it, though less frequently, in artificially fed infants. He also isolated it in the superficial ducts of the

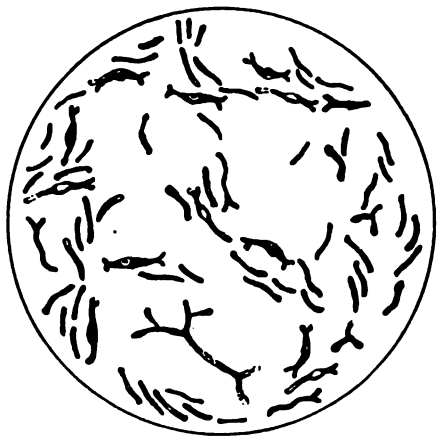
[1] Helen Baldwin has just shown in the case of one man that when milk plus a lactic acid bacillus was added to a mixed diet, the ethereal sulphates in the urine were increased.

mammary gland of the mother. It is a strict anaërobe. In the fæces and fresh cultures it presents the form of a slender bacillus with one end tapering and the other club-shaped. It varies in length from 2μ to 3μ and even 4μ.

It occurs mostly as a diplobacillus (see Fig. 94) with the pointed ends adjacent and the swollen ends free, but at times this order is reversed and a fusiform appearance results. As the line of separation is often obscure and as the two organisms come together at different angles these various arrangements give the impression of many different forms.

The bacilli lie sometimes in parallel groups, but are seldom entangled. In old cultures, the swollen ends seen in the young cultures become bifurcated, others take the stain irregularly and Tissier designates this the vesicular form. In some instances several bacilli become grouped together at different angles, giving the appearance of multiple branching forms. As the medium becomes more acid, the bifurcated forms become more numerous. Vesicular forms bear a relation to vitality and bifurcated forms a relation to media. It is

FIG. 94



B. Bifidus, representing the various forms described; the irregularly-stained or vesicular forms being from old cultures. × about 1800 diam.

non-motile, stains by Gram's method, old cultures staining irregularly. Does not seem to possess spores. Killed at 60° for 15 minutes. Does not die out quickly. Can be transplanted after three weeks. Grows best at 37° C., but also grows at 20° C.

On glucose agar, after three days, fine regular colonies, oval in shape, appear. It is innocuous to guinea-pigs. It can be cultivated on beerwort agar and on glucose agar. In stab cultures made from the fæces, in either medium the bacilli may be found in almost pure cultures at the bottom of the stab after two to fifteen days; the other organisms dying out unless the enterococcus, which is a facultative anaërobe, is present, then *B. bifidus*, being less adaptable, is overgrown. Fermentation tubes of glucose broth inoculated with fæcal material will show an abundant growth of *bifidus* in the sediment.

Conradi and Kurpjuweit claim that in infants the *B. bifidus* plays the same rôle in inhibiting the growth of the more harmful organisms as the *B. coli* does in the adults. Cahn states that the *B. bifidus* is

not found so constantly in artificially fed infants, but that the *B. acidophilus* of Mora takes its place.

B. Acidophilus.—This organism belongs to the acidophile group and differs from the bifidus in several respects. It never shows the bifid forms. It is only found in the artificially fed infants and in milk from cows. Colonies are irregular and send out filaments. It is a facultative anaërobe. Some consider it to be the same as the *B. bifidus*.

Enterococcus.—Thiercelin in 1903 described the *Enterococcus proteiformis* (Fig. 95) as occurring as a coccus, diplococcus, streptococcus, staphylococcus, tetrad filaments and rods. It has a capsule barely visible, sometimes forming a halo.

FIG. 95

Represents the gradation of the Enterococcus (Thiercelin) from the apparent bacillary forms to the coccus without a capsule. ×1000 diam.

The arrangement depends upon the mode of divisions. When the organism assumes the form of a bacillus the division takes place in line of the short axis, the capsule being tough does not rupture but encloses 2 or 4 more organisms. This form is observed in culture media containing alcohol, quinine, chromic acid, permanganate, and especially bichromate of potash. This bacillary form, before division takes place, is confusing as it is difficult to tell whether or not the culture is pure until transferred to a medium in which alcohol is present when the forms all become coccal. In strongly alkaline broth it grows in tetrads, on agar it resembles the staphylococcus, on gelatin the same. In broth containing a little methylene blue, picric or acetic acid and in hay infusions it is a distinct streptococcus. It is present in normal stools but may become pathogenic. It is found in the upper respiratory tract, skin, vagina, and is obtained in pure cultures from purulent discharges, being easily isolated by ordinary methods.

It is sensitive to heat and direct sunlight, rather resistant to disinfectants, does not grow in distilled water and sparingly in broth containing 2 per cent. sodium carbonate or nitrate. Grows in sterilized tap water, does not ferment sugars, does not constantly coagulate

milk, does not liquefy gelatin, grows well in the digestive fluids, no gas, indol, or odor in the presence of sugar.

It produces a toxin that kills mice in 24 to 48 hours. The organisms are found in all the organs after death.

B. Putrificus.—Bienstock found in putrid mixtures an anaërobic, spore-bearing bacillus resembling tetanus in its morphology, which is capable of decomposing fibrin in the absence of oxygen, in this case the end products of putrefaction, such as indol, are not formed. When, however, *B. putrificus* is associated with some aërobes it acts upon fibrin in the presence of oxygen, forming the characteristic putrefactive products, which are further split up by the aërobes, forming indol. This action is not observed with all aërobes, for example, with *B. coli* and *B. lactis aërogenes* inoculated on fibrin with *B. putrificus*.

B. putrificus is found commonly in the small intestines where it enters through the respiratory and digestive tract; that putrefaction does not occur, normally is supposed to be due to the presence of inhibiting bacteria. It is isolated with difficulty from the fæces. Is an obligate anaërobe with drum-stick spores. It is a slender rod with blunt ends, and sometimes forms threads, especially on liquid gelatin, is actively motile with flagella arranged on either side. Liquefies blood serum with the production of a foul odor. Is Gram-positive. Is not pathogenic for animals.

B. Aërogenes Capsulatus (*B. welchii*, *B. perfringens*).—Found usually in small numbers in healthy adults. Increased in old age. It is considered by Herter to be the chief cause of intestinal putrefaction. For a full description of its pathogenicity and other characteristics, see p. 440.

Klein found in the fæces of patients during an outbreak of diarrhœa at St. Bartholomew's Hospital, London, an organism which he named the *B. aërogenes sporogenes*, which may be a variety of *B. welchii*. (See also p. 442.)

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CHAPTER XX.

THE COLON-TYPHOID GROUP OF BACILLI.

THE COLON BACILLUS GROUP.

THERE are a number of varieties of bacilli occupying the intestines of man and animals which, because they have similar characteristics and live in the colon, are generally grouped together as colon bacilli. These bacilli are only pathogenic under unusual conditions. The specific pathogenic typhoid, paratyphoid, dysentery, and paradysentery bacilli, and those responsible for meat poisoning also have among themselves and between them and the colon bacilli resemblances and are often classed together in the group of the colon-typhoid bacilli.

The chief common characteristics of this whole group are: (1) a similar morphology, *i.e.*, short, rather plump rods with a tendency to thread formation; (2) a Gram-negative staining reaction; (3) similar growths on agar and gelatin; (4) non-liquefaction of gelatin (a few organisms which might be placed in the colon group, such as *B. cloacæ*, liquefy gelatin very slowly).

In order to see more clearly the main points of difference between the subdivisions of this great group the tabulation on page 256 may be studied.

The chief differential points between the individual species may be seen in the table on page 257.

The Bacillus Coli Group.—The first description of an organism of the colon type was by Emmerich (1885), who obtained it from the intestinal discharges of cholera patients. A similar organism was found by Escherich (1886) in the fæces of healthy infants. He gave it the name of *Bacterium coli commune*. It has since been demonstrated that closely allied types of bacilli are normal inhabitants of the intestines of most of the lower animals. They are transferred through the fæces as manure and sewage to cultivated land, surface waters, etc. During warm weather they may increase outside of the animal body. Those strains having the chief cultural characteristics of the original strain are classed as colon bacilli, while those differing considerably from it are, while considered in the general group, given different names, such as paracolon, etc.

The group of the *B. coli* has interest not only because it excites disease at times in man and animals, but also because it is an index of fæcal pollution from man or animals. If from man it indicates the possibility of infection with the typhoid or dysentery bacilli.

Morphology.—*Bacillus coli* varies considerably in its morphology, according to the sources and the culture media from which it is ob-

Group of Colon-typhoid Bacilli.

B. Coli group. Also called the group of lactose fermenters. Motility generally not marked or none. Dextrose and lactose fermented with gas formation. Milk quickly coagulated with acid production. Indol produced by most varieties.	B. Coli (many varieties). B. [lactis] aerogenes. B. [mucosus] capsulatus. (B. pneumoniæ). B. of rhinoscleroma. B. Cloacæ (?).	Generally capsulated and non-motile.	Frequently non-pathogenic for man, but may become distinctly pathogenic under certain conditions. Varying degree of virulence for lower animals upon inoculation.
B. Enteritidis group. Often called the *group of Intermediates.* Motility usually marked. Dextrose fermented with gas formation. Lactose not fermented. Milk not coagulated. No indol or only slight amount produced. (B. alcaligines ferments no sugars.)	B. enteritidis (many var.). B. psittacosis. B. icteroides. B. paracoli. B. paratyphosus. B. of hog cholera. B. of swine plague. B. alcaligenes.		Under certain conditions most members distinctly pathogenic for many of the lower animals and for man.
B. Dysenteriæ group. Often grouped with B. typhosus. Non-motile. Dextrose fermented without gas formation. Lactose not fermented. Milk not coagulated. No indol formed. Mannite not fermented in B. dysenteriæ. Maltose and mannite fermented in B. paradysenteriæ, B, mannite only in A.	B. dysenteriæ. B. paradysenteriæ, A (Park). B. paradysenteriæ, B (Flexner).		Pathogenic for man, the first more than the others. Less pathogenic for lower animals (inoculations).
B. Typhosus group. Actively motile. Dextrose fermented without gas formation. Lactose not fermented. Milk not coagulated. No indol formed.	B. Typhosus.		Pathogenic for man. Less pathogenic for lower animals (inoculations).

Chief Differential Points Between Species of the Colon-typhoid Group.

Organism	Motility	Flagella	Indol production	Sugars: Dextrose	Lactose	Maltose	Dextrin	Saccharose	Mannite	Amount gas produced	Milk	Neutral red	Potato	Uschinsky	Colonies on plate media: Conradi Drigalski	Endo
B. Coli	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. Coli strain A	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. Coli strain B	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. Coli strain C	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. Coli strain D	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. Coli strain E	+	+	+	+	+	+	+	+	+	− +	+	+	+	+	Red	Red
B. (Lactis) aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Red	Red
B. (mucosus) capsulatus (B. pneumoniæ)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Blue	Colorless, transparent
B. rhinoscleromæ	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Blue	Colorless transparent
B. cloacæ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Red	Red
B. enteritidis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Red	Red
B. psittacosis	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. icteroides	−	−	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. paracoli	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. paratyphosus, A	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. paratyphosus, B	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. of hog cholera	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. of swine plague	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. alcaligenes	+	+	−	+	−	+	+	+	+	+	+	+	+	+	Red	Red
B. dysenteriæ (Shiga)	−	−	+	×	−	×	×	−	×	−	×	−	+	−	Blue	Colorless transparent
B. paradysenteriæ, A (Park)	−	−	+	×	−	×	×	−	×	−	×	−	+	−	Blue	Colorless transparent
B. paradysenteriæ, B (Flexner)	−	+	−	×	−	×	×	−	×	−	×	−	+	−	Blue	Colorless transparent
B. typhosus	+	+	−	×	−	×	×	−	×	−	×	−	+	−	Blue	Colorless transparent

\+ Acid and gas (sugars), acid and clot (milk).
× Acid without gas (sugars), or acid without clot (milk).
\+ Scant.
++ Abundant.
+− Moderate.
− Blanks mean that no work has been reported on those points.

Group of Colon-typhoid Bacilli.	*B. Coli group.* Also called the group of lactose fermenters. Motility generally not marked or none. Dextrose and lactose fermented with gas formation. Milk quickly coagulated with acid production. Indol produced by most varieties.	B. Coli (many varieties). B. [lactis] aerogenes. B. [mucosus] capsulatus. (B. pneumoniæ). B. of rhinoscleroma. B. Cloacæ (?).	Generally capsulated and non-motile.	Frequently non-pathogenic for man, but may become distinctly pathogenic under certain conditions. Varying degree of virulence for lower animals upon inoculation.
	B. Enteritidis group. Often called the *group of Intermediates.* Motility usually marked. Dextrose fermented with gas formation. Lactose not fermented. Milk not coagulated. No indol or only slight amount produced. (B. alcaligines ferments no sugars.)	B. enteritidis (many var.). B. psittacosis. B. icteroides. B. paracoli. B. paratyphosus. B. of hog cholera. B. of swine plague. B. alcaligenes.		Under certain conditions most members distinctly pathogenic for many of the lower animals and for man.
	B. Dysenteriæ group. Often grouped with B. typhosus. Non-motile. Dextrose fermented without gas formation. Lactose not fermented. Milk not coagulated. No indol formed. Mannite not fermented in B. dysenteriæ. Maltose and mannite fermented in B. paradysenteriæ, B, mannite only in A.	B. dysenteriæ. B. paradysenteriæ, A (Park). B. paradysenteriæ, B (Flexner).		Pathogenic for man, the first more than the others. Less pathogenic for lower animals (inoculations).
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Chief Differential Points Between Species of the Colon-typhoid Group.

Organism	Motility	Flagella	Indol production	Sugars: Dextrose	Lactose	Maltose	Dextrin	Saccharose	Mannite	Amount gas produced	Milk	Neutral red	Potato	Uschinsky	Colonies on plate media: Conradi Drigalski	Endo.
B. Coli	±	±	+	+	+	+	+	±	+	−	+	+	+	+	Red	Red
B. Coli strain A	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. Coli strain B	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. Coli strain C	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. Coli strain D	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. Coli strain E	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. (Lactis) aerogenes	+	+	+	+	+	+	+	±	+	+	+	+	+	+	Red	Red
B. (mucosus) capsulatus (B. pneumoniæ)	−	−	−	+	+	+	+	+	+	+	×	−	−	+	Red	Red
B. rhinoscleromæ	−	−	−	+	+	+	+	+	+	+	+	−	−	+	Red	Red
B. cloacæ	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. enteritidis	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. psittacosis	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. icteroides	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. paracoli	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. paratyphosus, A	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. paratyphosus, B	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. of hog cholera	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. of swine plague	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. alcaligenes	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Red	Red
B. dysenteriæ (Shiga)	−	−	+	×	−	×	×	−	−	−	×	−	+	+	Blue	Colorless, transparent
B. paradysenteriæ, A (Park)	−	−	+	×	−	×	×	−	−	−	×	−	+	+	Blue	Colorless transparent
B. paradysenteriæ, B (Flexner)	−	−	+	×	−	×	×	−	−	−	×	−	+	+	Blue	Colorless transparent
B. typhosus	+	+	−	×	−	×	×	−	−	−	×	−	+	+	Blue	Colorless transparent

\+ Acid and gas (sugars), acid and clot (milk).
× Acid without gas (sugars), or acid without clot (milk).
± Scant.
++ Abundant.
+ | Moderate.
Blanks mean that no work has been reported on those points.

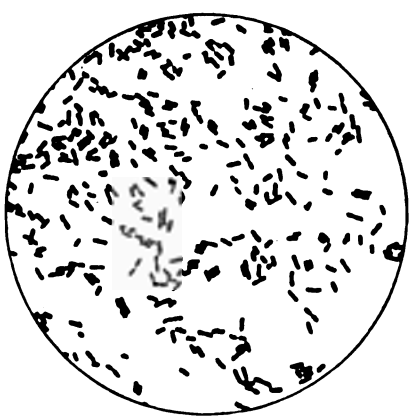
tained. The typical form (Fig. 96) is that of short rods with rounded ends, from 0.4μ to 0.7μ in diameter by 1μ to 3μ in length; sometimes, especially where the culture media are not suitable for their growth and in tissues, the rods are so short as to be almost spherical, resembling micrococci in appearance, and, again, they are somewhat oval in form or are seen as threads of 6μ or more in length. The various forms may often be associated in the same culture. The bacilli occur as single cells or as pairs joined end-to-end, rarely as short chains. There is nothing in the morphology of this bacillus sufficiently characteristic for its identification.

Flagella.—Upon some varieties seven or eight peritrichic flagella have been demonstrated, upon others none. The flagella are shorter and more delicate than those characteristic of the typhoid bacilli.

Staining.—The colon bacillus *stains* readily with the ordinary aniline colors; it is always decolorized by Gram's method. Under certain conditions the stained bacilli exhibit bipolar granules.

Biology.—It is an *aërobic, facultative anaërobic, non-liquefying* bacillus. It develops best at 37° C., but grows well at 20° C., and slowly at 10° C. It is usually motile, but the movements in some of the cultures are so sluggish that a positive opinion is often difficult. In fresh cultures, frequently, only one or two individuals out of many show motility. MacConkey recommends examining under dark ground illumination a drop of a 6-hour broth culture placed on an ordinary glass slide without a coverslip, using a $\frac{1}{2}$-inch objective and a $\times 8$ eye-piece. This gives a good idea of the power of movement of an organism.

FIG. 96



Colon bacilli. Twenty-four-hour agar culture. × 1100 diameters.

The *B. coli* does not form spores.

Cultivation.—The colon bacillus develops well on all the usual culture media. Its growth on them is usually more abundant than that of the typhoid bacillus or the dysentery bacillus, but the difference is not sufficient for a differential diagnosis.

Gelatin.—In gelatin plates, colonies are developed in from eighteen to thirty-six hours. They resemble greatly the colonies of the typhoid bacillus, except that many of them are somewhat larger and more opaque. (See Figs. 42–44, page 73.) When located in the depths of the gelatin and examined by a low-power lens they are at first seen to be finely granular, almost homogeneous, and of a pale yellowish to brownish color; later they become larger, denser, darker, and more coarsely granular. In shape they may be round, oval, or whetstone-like. When the gelatin is not firm the margins of many

colonies are broken by outgrowths, which are rather characteristic of colon bacilli.

In stab cultures on gelatin the growth usually takes the form of a nail with a flattened head, the surface extension generally reaching out rapidly to the sides of the tube.

Nutrient Agar.—In plate cultures: Surface colonies mostly circular, finely granular, and rather opaque. The deep colonies are apt to have protuberances. In streak cultures an abundant, soft, white layer is quickly developed, but the growth is not characteristic.

Bouillon.—In bouillon the *Bacillus coli* produces diffuse clouding with sedimentation; in some cultures a tendency to pellicle formation on the surface is occasionally seen.

Potato.—On potato the growth is rapid and abundant, appearing after twenty-four to thirty-six hours in the incubator as a yellowish-brown to dark cream-colored deposit covering the greater part of the surface. But there are considerable variations from the typical growth; there may be no visible growth at all, or it may be scanty and of a white color. These variations are due often to variations in the potato.

Milk.—Milk coagulates in from four to ten days at 20° C., and in from one to four days at 37° C. The acids formed are lactic, acetic, formic, and succinic acids. Coagulation is due principally but not altogether to acids. A ferment is produced which is capable of causing coagulation in the presence of lime salts, especially in acid solutions.

Chemical Activities.—Behavior Toward Carbohydrates.—In cultures of colon bacilli many carbohydrates, especially sugars, become fermented with production of acid and gas.

There are differences, however, among the organisms thus included under the name colon bacilli; thus, some ferment cane-sugar, others do not. The great majority ferment glucose and lactose with the production of gas.

The important fermentation products, both qualitatively and quantitatively, are produced from grape-sugar, probably, according to the following reaction:

$$\underset{\text{Grape-sugar.}}{2C_6H_{12}O_6} + \underset{\text{Water.}}{H_2O} = \underset{\text{Lactic acid.}}{2C_3H_6O_3} + \underset{\text{Acetic acid.}}{CH_3COOH} + \underset{\text{Ethyl alcohol.}}{C_2H_5COOH} + \underset{\text{Carbonic acid.}}{2CO_2} + \underset{\text{Hydrogen.}}{2H_2}$$

There are reasons to think that lactic acid is first produced and that from this other acids and products develop. Under aërobic conditions lactic is produced in excess of acetic acid, while in the absence of oxygen the reverse is apt to be true.

Gas Production.—When colon bacilli are grown in a solution of glucose (dextrose), CO_2 and H_2 are produced, $1CO_2$ to $1H_2$ up to $1CO_2$ to $3H_2$. Anaërobic conditions aid gas formation. A very few intestinal varieties of Gram negative bacilli produce gas from no sugars and some from a few only. Nearly all produce gas from glucose, and about 80 per cent. of all varieties produce gas from milk-sugar (lactose). Very slight traces of gases other than H and CO_2 are

produced. The amount of gas varies in different varieties; the closed arm of the tube half-filled, and the H and CO_2 in the proportion 2 to 1, is the characteristic type. It is also true of Gärtner's *B. enteritidis*. In another type the whole of the closed arm is filled,—$H_2 : CO_2 = 1 : 2$ or 3. This type is usually called *Bacillus cloacæ*. In a third type the arm is nearly filled, $-H_2 : CO_2 = 1 : 1$. This type is the *B. aërogenes*.

The fermentation is not a simple hydrolytic action, but one in which combinations between the C and O atoms are sundered and formed. This is not an oxidation process, but a change through breaking down—that is, a true decomposition. What oxidation takes place is chiefly due to the oxygen liberated from splitting the sugar molecules.

USE OF NEUTRAL SUGAR LITMUS AGARS TO DIFFERENTIATE BETWEEN COLON AND TYPHOID BACILLI.

List of sugars.	Color of media after 24 hours' growth of culture.	
	Colon bacillus.	Typhoid bacillus.
Grape-sugar (Dextrose)	Red.	Red.
Saccharose	Red or blue.	Blue.
Mannite	Red.	Red.
Maltose	Red or moderately red.	Red or pink.
Milk-sugar (Lactose)	Red.	Blue.
Dextrin	Blue	Violet blue.

To bouillon used to detect acid and gas formation no sodium hydrate or carbonate should be added.

Effect of Colon Bacilli in Nitrogenous Compounds.—Indol Formation.—None of the Colon bacilli liquefy gelatin nor peptonize any albumins. They do, however, break down some of the higher nitrogenous compounds into smaller atom groups. The first noted of these compounds was indol, $C_6H_4\langle^{NH}_{CH}\rangle CH$. This is one of the most important products of colon activity, although a few varieties lack it. (Witte's peptone solution is used to develop indol.) Sugars interfere with its production, as also does the absence of oxygen. The maximum amount of indol is present about the tenth day. In the intestinal canal in health very little indol appears to be produced by colon bacilli. Sulphurated hydrogen is liberated from sugar-free fermentable proteid substances. Mercaptan and sometimes skatol have been noted in peptone solution cultures. The colon bacillus liquefies, according to some minute quantities of gelatin, but so little as to be inappreciable.

In media containing fermentable sugars and proteid substances simultaneous action takes place on both with the production of both alkalies and acids.

Test for Indol.—The test is carried out by adding little by little ½ to 2 c.c. of 0.005 per cent. potassium nitrite to 10 c.c. of fluid culture, to which has been added 5 c.c. of 10 per cent. sulphuric acid. To prevent confusion with other colors a little amyl alcohol is added and shaken to dissolve and concentrate the color.

Effect on Fats.—No action has been noted.

Reduction Processes.—Reduction of Pigments.—The action on certain pigments which are reduced to colorless products and intermediate colors is more vigorous than that of typhoid bacilli. This effect occurs in litmus bouillon in the closed arm of the tube, in bouillon and agar (not in gelatin), indigo-sodium-sulphate-methyl blue, in sugar media, etc.

Reduction of Inorganic Salts.—From nitrates the bacilli produce nitrites and from them ammonia and free nitrogen as follows:

$$2NaNO_3 + H_2O = 2NaOH + N_2 + O_5.$$

Toxins.—The bodies of dead colon bacilli contain pyogenic substances (and others), which, injected into the circulation, produce paralysis of the striped muscle fibres, convulsions, coma, and death. Extracts from some cultures produce irritation of the mucous membranes of the large intestines with dysenteric symptoms.

Growth with Other Bacteria.—The colon bacilli act antagonistically to many of the proteolytic bacteria in the intestinal tract, and so inhibit alkaline putrefaction otherwise caused by the latter. In milk the same antagonism exists, probably because of the acidity caused by the colon growth.

Reaction to High and Low Temperatures.—Colon bacilli are killed at 60° C. in from five to fifteen minutes. Frozen in ice a large proportion die, but some resist for six months. Frozen in liquid air 95 per cent. are killed in two hours.

Resistance to Drying and Antiseptics.—Simple drying destroys the majority of organisms dried at any one time, but some bacilli of the number dried may remain alive, especially when held in the texture of threads, for five or six months, or all may die in forty-eight hours.

To most antiseptics they are moderately resistant. They are killed in 5 to 15 minutes in a 1 per cent. solution of carbolic acid.

Effect of Acids.—The colon bacilli grow in a wider range of acids and alkalies than most other bacteria. They develop in from 0.2 to 0.4 per cent. of mineral acids, in from 0.3 to 0.45 per cent. of vetegable acids, and in from 0.1 to 0.2 per cent. of alkalies.

Effect of Animal Fluids and Juices.—Gastric juice kills colon bacilli when not protected or too greatly diluted by food. All the members of the typhoid-colon group are more resistant to the gastric juices than most non-spore-bearing bacteria. With the food they readily pass from the stomach into the intestines. They grow in bile and in the intestinal juices.

Occurrence in Man and Animals during Health.—The *Bacillus coli* is a common inhabitant of the intestinal canal in man and in almost all domestic animals. Many of the varieties found in animals seem to be identical with those found in man. It is also found occasionally in wild animals and appears at times even to occur in fishes.

Occurrence Outside of the Intestines.—Colon bacilli are found wherever human or animal fæces are carried. They are therefore

present in all cultivated soil and inhabited country. In surface water a few bacilli, less than one to each cubic centimeter, are not sufficient to give rise to the opinion that it is contaminated. Even the presence of ten colon bacilli in 1 c.c. does not necessarily show dangerous pollution, since such a number could equally well come from the rain-water from streets or fields. Inspection alone can hope to reveal the source of the bacilli and therefore their significance. Colon bacilli are apt to be found in everything which comes in contact with man or animals.

Association with Other Bacteria in the Intestines.—In the breast-fed infant within a few hours or days after birth one or two varieties of typical colon bacilli are found in the colon, and these bacilli form the great majority of all the bacteria present *which grow in media*. The bacilli in one infant's intestines usually all agglutinate with the same serum, but those from different infants vary. The bacilli find their way through the food or from the anus upward. In the small intestines the *Bacillus aërogenes* is most prevalent, while in the cæcum and below the characteristic colon types predominate.

Only about 10 per cent. of the bacteria from stools seen under the microscope appear as colonies, and whereas in infant stools the majority of the bacteria in spreads are frequently Gram-positive, the larger number of the colonies are composed of Gram-negative bacteria. Some of the Gram-positive bacteria are anaërobic; others fail to grow at all on ordinary culture media. These conditions, the normal presence of colon bacilli and the tendency of other bacteria not to grow in culture media, make the greatest care necessary in weighing conclusions as to the pathogenic significance of colon bacilli in disease.

Pathogenesis.—In Lower Animals.—Intraperitoneal and intravenous inoculation of guinea-pigs and rabbits may produce death, which, when it follows, usually takes place within the first forty-eight hours, accompanied by a decided fall of temperature, the symptoms of enteritis, diarrhœa, etc., and finally fibropurulent peritonitis.

Subcutaneous inoculation in rabbits is followed usually by abscess formation at the point of inoculation. Dogs and cats are similarly affected.

Albarran and Hallé have caused cystitis and pyelonephritis by direct injections into the bladder and ureters, the urine being artificially suppressed; Chassin and Roger produced angiocholitis and abscess of the liver in the same way. Akermann produced osteomyelitis in young rabbits by intravenous injections of cultures.

From experiments on animals it would, therefore, appear that the true explanation of the pathogenesis of the colon bacillus is undoubtedly to be found in the toxic effects of the chemical substance and products of the cells.

In Man.—In normal intestines with intact mucous membranes the toxic products formed by the colon bacilli are absorbed but little or not at all, and the bacilli themselves are prevented from invading the tissues by the epithelial layer and the bactericidal properties of the

body fluids. Possibly there is an acquired immunity to the colon varieties which have long inhabited the intestines.

The colon bacillus was at first regarded purely as a saprophyte. Later, through not realizing the post-mortem invasion and the great ease of growth of the colon bacillus on ordinary media, the other extreme was taken of attributing too much to it.

The bacilli previously present in the intestines can, either by an increase in virulence in them or by a lowered resistance in the person, invade the tissues in which their toxins act, causing injury to the intestinal tract. Thus in the case of ulceration in typhoid fever the colon bacilli enter the blood, or in perforation produce peritonitis. In dying conditions they at times pass through the intact mucous lining. In the gall-bladder or urinary tract the spread of bacilli from the intestines may cause disease. The specific serum reaction in the body is a sign of infection, but great care has to be observed in deciding that it is present, as group agglutinins also occur. Up to the present time it is very difficult to state in any colon infection whether the bacilli were previously present in the intestines or were derived from outside sources through water, food, or direct contact with other cases.

Intestinal Lesions.—The lesions present in intestinal infection are those of enteritis; the duodenum and jejunum are found to contain fluid, the spleen is somewhat enlarged, and there are marked hyperæmia and ecchymosis of the small intestines, together with swelling of Peyer's patches.

Virulence of Colon Bacilli from Normal and Diseased Intestines.—The virulence varies with the culture and the time since its recovery from the intestines. Other things being equal, it is more virulent from an intestinal inflammation. From severe diarrhœa the colon bacilli in 0.25 c.c. bouillon culture may kill guinea-pigs if given intraperitoneally, while from the healthy bowel 2.5 c.c. are usually required.

Increase of Virulence Outside the Body.—It has been found by several observers that in fermenting fæcal matter a marked increased virulence takes place, so that infection is produced when received by man.

Colon Bacillus in Sepsis.—In lesions of the intestinal mucous membranes or in colon cystitis, pyelitis, or cholecystitis, there is frequently just before death a terminal dissemination of the bacilli and consequent septicæmia. Here special symptoms of intoxication may occur, such as diarrhœa, changes in temperature, heart weakness, and hemorrhages. In most of these cases infection proceeds from the intestines, but in not a few from the wounded urethra or bladder. The colon septicæmia is detected by blood cultures. At times very few bacilli are found, and then the blood infection may be less important than the local one. Cases occurring in typhoid and cholera are often observed, especially in relapses in typhoid. In very young infants a malignant septicæmia with tendency to hemorrhages is due to colon septicæmia. In a few cases in which colon but no typhoid

bacilli were present the course of the disease has been similar to typhoid fever. An epidemic due to colon infection of water has been noted. Infection through food and water are usually brought about by other closely allied bacilli not belonging to the colon group.

Colon Bacillus in Diarrhœa.—In diarrhœa we find increased peristalsis, less absorption of foodstuff, increased and changed intestinal secretions. Tissier observed that under treatment with cathartics the colon varieties increased, while the anaërobic forms are inhibited. In diarrhœa exciting conditions are active, inhibiting causes are lessened, and increased mucus and serum are poured out into the canal. This is notably seen in typhoid fever. In diarrhœa, although the common colon varieties are met with, there is usually seen a difference in that uncommon varieties and more typhoid-like bacteria are also found. Much more investigation is needed on this complex subject of variation in types between health and disease.

Lesage, in 1898, stated that 25 per cent. of 770 cases reported by him of breast-fed children were due to pure colon infection, while the others were from mixed infection in which the significance of the colon bacilli present was more doubtful, as there are other, slightly different, microörganisms, colon-like in their characteristics, which produce infection. The reasons for this opinion are given by Escherich and Pfaundler as follows:

1. Animals are certainly affected by epidemic infections of bacteria closely allied to the colon group—*e. g.*, diarrhœa of calves and cows, hog-cholera, enteritis with ulceration in horses, etc.

2. The histories of attacks of acute diarrhœa in men after eating food of such infected animals, and the presence of the serum reaction afterward. These bacteria are colon-like, though classed with the enteritidis group.

3. The diseases of typhoidal nature are due to the closely allied paracolon or paratyphoid bacilli, and others are due to the dysentery group, in which the inflammatory and necrotic process localizes itself mostly in the lower colon and rectum.

Numerous epidemics of acute diarrhœa in children from one to five years of age have been noted in which almost pure cultures of colon bacilli have been found. The symptoms begin with high fever which often rapidly falls, and frequent stools only watery or containing mucus and streaks of blood. These symptoms may quickly abate or go on to a toxic state characterized by heart weakness and drowsiness. This may lead to lung complications or death. In many such cases in America when blood has been present we have found one of the mannite fermenting types of the dysentery bacillus.

B. Coli in Peritonitis.—Here the lesions must be considered as being due to mixed infection.

Experimental evidence goes to show that the injection of virulent cultures of any of the varieties of colon bacilli into the peritoneal cavity produces intense and fatal peritonitis. Not only perforation of the intestines in man, but injury to the intestinal walls, allows colon infec-

tion of the peritoneum to take place, and if foreign bodies are present in the peritoneum, or the epithelium injured, or absorption interfered with, such acute general peritonitis is very probable. At first most of these cases were believed to be a pure colon infection, but now it is known that this idea came largely from the overgrowth of colon bacilli in the cultures. More careful investigations, through cultures and smears, have demonstrated the fact that streptococci, and less frequently staphylococci and pneumococci, are also usually present in peritonitis arising from intestinal sources. The colon bacilli found even in the same case commonly comprise many varieties.

The Colon Group in Inflammation of the Bile Tract.—The normal healthy gall-bladder is usually sterile. This is true in spite of the fact that bile is apparently a good culture medium for the colon group. Simple tying of the neck of the gall-bladder usually causes a colon infection to take place within twenty-four hours. Obstruction of the bile-duct through various causes is fairly common in man. The gall-bladder then becomes infected, and following the inflammation of the mucous membranes there is often the formation of gall-stones. Some cases of jaundice are believed to be due to colon inflammation of the gall-ducts. Atypical varieties of *B. Coli* are frequently isolated from gall-bladder infections.

Inflammation of the Pancreas.—Welch was the first to record a case of pancreatitis with multiple fat necroses due to colon infection. A few more cases have since been reported due to members of the colon group, either alone or in conjunction with the pyogenic cocci.

Inflammation of the Urinary Tract.—As far back as 1879 Bouchard noted cystitis due to bacilli of the colon group. After injury of the bladder mucous membrane, or by ligature of the urethra, it is possible to excite cystitis in animals by injection of colon bacilli. When cystitis is established the bacterial infection frequently spreads to the pelvis of the kidneys, causing a pyelitis or suppurative nephritis. The same process often occurs in man. In most cases of chronic cystitis the ureters and pelves of the kidneys become involved; any malformation of the ureters aids the process. From the pelvis the bacteria push up into the urinary tubules and excite inflammation and multiple abscesses. Colon infection of the different parts of the urinary tract may occur at any age, from infancy upward. Instead of starting in the bladder it may begin in the kidney itself, the colon bacilli coming from the blood or peritoneum. In many of these cases the bacilli isolated from the urine are clumped in high dilutions of the blood from the patient.

Although other bacteria—the pyogenic cocci, the proteus, the typhoid bacillus, etc.—may excite cystitis, still in 90 per cent. of all cases some of the colon group are found, and this percentage is even higher in young children. The clinical picture of colon infection is very variable. The lightest cases progress under the guise of a bacteriuria. The urine is passed a little more frequently and shows a

fine granular cloudiness. The reaction is acid. The cell elements are but little increased. There is an excess of mucus. Albumin is absent or present in only a trace. The condition may last for weeks or months and then spontaneously disappear or grow worse. With a somewhat more severe infection there is painful urination, perhaps tenesmus, increase of pus cells and slight fever. In a conical glass a sediment of pus cells forms at the bottom, and clear urine remains above. If the infection passes to the kidney colicky pain and tenderness over the region of the kidneys is usually present. The most important symptom of pyelitis is an irregular intermittent fever resembling malaria. The albumin is increased in the urine and red blood cells may be seen. If a general nephritis arises the symptoms are all intensified and an anæmic condition may develop. Septicæmia may finally result.

In most of these cases the microscopic examination is sufficient to make a probable diagnosis, since the bacteria are so abundant. The variety of colon bacillus present can, of course, only be told by cultures and other means. In the urine they appear as diplobacilli, or partly in short, almost coccus, forms, partly in long threads. As a rule, motility is absent. Not infrequently the cultures appear to be identical with those of the *Bacillus aërogenes*.

The characteristics of the urine itself have much to do with the probability of infection; the more acid urines being less likely to afford a proper soil for growth. Some urines are bactericidal even when they are neutral. The substances producing this condition are not known. The colon bacilli in the urine produce no appreciable effect on the reaction, but give up some of their toxins, which upon absorption cause the deleterious local and general effects. The serum of the patient usually agglutinates the cultures from the urine in 1: 20 or 1 : 50 dilutions, but this property is sometimes absent, especially in light cases.

In all cases in addition to the introduction of the colon bacillus a predisposing condition must be present, such as more or less marked retention of urine by an enlarged prostate or stricture, any unhealthy state of the mucous membrane or general depression of vitality.

The B. Coli as Pus Formers.—Members of this group are frequently the cause of abscesses in the region of the rectum, urethra, and kidney. They rarely produce pus in other locations.

The Colon Group in Inflammation Not Previously Mentioned.—Broncho-pneumonia, lobar pneumonia, and pleurisy have occasionally been caused by colon bacilli, probably from blood sources. Not a few cases of meningitis and spinal meningitis in infants, following localized colon infections, are due to colon bacilli. The symptoms are not well developed, as a rule. Some cases of endocarditis have also been noted.

Treatment.—Prophylactic.—Immunization against colon bacillus infection can be produced, as in typhoid bacillus infection, by giving one injection of 300 millions followed in ten days by 500 millions.

The serum can be prepared but is not at present employed therapeutically. It would have to be made by injecting horses with many different strains.

Curative Vaccine Treatment.—Localized inflammations due to the *B. coli* have been treated quite successfully by injections of dead organisms. An injection of 25 millions can be made daily or 150 to 300 millions every three to seven days. Autogenous vaccines should always be prepared if possible.

Methods of Isolation.—While the isolation of typhoid bacilli from fæces, water, dust, etc., is attended, as a rule, with difficulty, pure cultures of colon bacilli can usually be obtained from such substances by the simplest procedures. The following methods may be recommended:

1. Inoculate 10 c.c. of fluid 2 per cent. lactose neutral litmus agar with diluted fæces or suspected material. The melted agar should be at a temperature of about 41° C. After shaking pour in Petri dish. Several dilutions should be made. After eighteen hours at 37° C. examine the plates and inoculate the contents of a number of tubes containing 2 per cent. lactose agar with any colonies showing a red color. The colon bacilli will produce gas and acid (see page 256).

2. Inoculation of increasing quantities of the material (water) in 2 per cent. dextrose nutrient bouillon and 2 per cent. lactose peptone. solution or lactose peptone bile contained in fermentation tubes. The presence of colon bacilli in the inoculated portion produces after twelve to twenty-four hours active fermentation.

Bacillus [Lactis] Aerogenes.—This organism resembles very closely the colon bacillus, and in ordinary tests is not differentiated from it. Furthermore, the two organisms are often found together in the intestines and in infections elsewhere. *B. aerogenese* is found frequently in milk (especially in sour milk) where it usually develops a capsule.

Another capsule-forming bacterium which may be placed in this group is

THE PNEUMOBACILLUS OF FRIEDLANDER.—B. PNEUMONIÆ, B. [MUCOSUS] CAPSULATUS.

This bacillus discovered by Friedländer (1883) is now known to occur frequently as a mixed infection in cases of phthisis, fibrinous pneumonia, and in rare instances as the only exciting factor in pneumonia. It is also not infrequently found in the mucous membranes of the mouth and air passages of healthy individuals.

Morphology.—Short bacilli with rounded ends, often resembling micrococci, especially in recent cultures; commonly united in pairs or in chains of four, and, under certain circumstances, surrounded by a transparent capsule. This capsule is not seen in preparations made from artificial culture media, but is visible in well-stained preparations from the blood of an inoculated animal.

Friedländer's bacillus *stains* readily with the aniline colors, but is not stained by Gram's method.

Biology.—An aërobic, non-motile, non-liquefying bacillus; also facultative anaërobic; does not form spores. In *gelatin stick cultures* it presents the "nail-shaped" growth first described by Friedländer, which is not, however, peculiar to this bacillus, and in old cultures the gelatin acquires a distinct brownish coloration. This latter characteristic distinguishes the growth of this bacillus from that of the *Bacillus aërogenes*, which is otherwise very similar to it morphologically. On *gelatin plates* colonies appear at the end of twenty-four hours as small white spheres, which rapidly increase in size. These colonies, when examined by a low-power lens, present a somewhat irregular outline and a slightly granular appearance. The growth on *agar* is in quite large and moist grayish colonies. The growth on *potato* is luxuriant—a thick, yellowish-white, glistening layer rapidly covering the entire surface. *Milk* is not coagulated. *Indol* is produced in bouillon or peptone solutions. Milk-sugar and glucose are fermented. Growth occurs at 16° to 20° C., but is more rapid at 37° C.

Pathogenesis.—Friedländer's bacillus is pathogenic for mice and guinea-pigs, less so for dogs, and rabbits are apparently immune. On autopsy after death due to inoculation into the lungs, the pleural cavities are found to contain a seropurulent fluid, the lungs are intensely congested, and in places show limited areas of red hepatization; the spleen is considerably enlarged, and bacilli are present in the lungs, the pleuritic fluid, and the blood.

Friedländer's bacillus has been found in man, not only in patients suffering from croupous pneumonia and other respiratory diseases, but in healthy individuals, and also in the outside world. Netter observed it in 4.5 per cent. of the cases examined by him in the saliva of healthy individuals, and Pansini in cases of pulmonary tuberculosis in the sputum. In 129 cases of pneumonia examined by Weichselbaum this bacillus was found in only 9. The cases which are due primarily to the pneumo-bacillus are distinguished, according to Weichselbaum and Netter, by their peculiarly malignant type and by the viscidity of the exudate produced. This bacillus is also probably concerned, primarily or secondarily, under certain circumstances, in the production of pleurisy, abscess of the lungs, pericarditis, endocarditis, otitis media, and meningitis, in all of which it has at times been found to be present. Vaccines have been used successfully in treatment.

The "*bacillus of rhinoscleroma*" (see Fig. 18), found frequently in the lesions of an infectious-granuloma type of disease in the nose, is very similar to the *B. capsulatus*. Some investigators do not consider it possible to differentiate the two organisms. Some believe that the bacilli found in rhinoscleroma are secondary invaders. The rhinoscleroma bacilli do not always produce gas in sugar media and they are only slightly pathogenic for experimental animals.

INTERMEDIATE MEMBERS OF THE TYPHOID-COLON GROUP OF BACILLI.

Gärtner's discovery in 1888 of the *Bacillus enteritidis*, in association with epidemics of meat poisoning, first gave impetus to the study of a number of parasitic bacteria resembling in many characteristics the colon or typhoid bacilli. These bacilli are fequently termed inter-

mediates. Nocard's work showing that *Bacillus psittacosis* caused infection in parrots followed in 1892. In 1893 Gilbert introduced the terms "paracolon" and "paratyphoid" to designate bacilli of this group resembling more nearly in biological characters the colon bacillus on the one hand and the typhoid bacillus on the other.

The intermediates include *Bacillus enteritidis* and similar organisms recovered from cases of epidemic meat poisoning, the gas-producing typhoid-like bacilli of various observers obtained from cases suffering from typhoidal symptoms, *Bacillus psittacosis*, *Bacillus choleræ suis* (hog-cholera), bacillus of swine plague, *Bacillus icteroides*, *Bacillus alcaligenes* (Tables, pp. 256, 257).

The paracolon and paratyphoid can be distinguished without difficulty from the typhoid bacilli. They produce gas in glucose media, and in this respect they differ from typhoid, but, unlike *Bacillus coli*, they do not produce gas from lactose, coagulate milk, or, as a rule, form indol.

The main points of difference between the two varieties are that the paracolons turn milk and whey alkaline after a short initial acidity and form gas freely in glucose media, while with the paratyphoids there is in milk and whey an initial acidity, but no or very slight subsequent alkalinity; the gas production in glucose media is much less pronounced. Neutral red agar also differentiates between the two groups. Like *Bacillus coli*, all the intermediates reduce the color to yellow in twenty-four to seventy-two hours, but with the paratyphoids after four or five days the red color begins to return from above downward until in two or three weeks the medium is again red throughout. With the paracolons the yellow color is permanent. (Refer to table pp. 255, 256, for chief differential points of whole group.)

Agglutination tests applied to the intermediates show that the members of the paracolon group do not all show mutual reactions, and the group must, therefore, be composed of a number of distinct races, as in the case with *Bacillus coli*. The paratyphoids, on the other hand, most of which have been isolated from cases simulating typhoid fever belong chiefly to two strains; that is to say, an active serum prepared from either strain of the bacilli will agglutinate all the others of that strain. These are designated as type A and type B.

Relative Frequency of Paratyphoid Infections.—Gwyn's case was the only one of 265 cases which failed to give Widal reaction. Schottmüller and Kurth from a total of 180 cases which had been looked upon as typhoid, were able in 12 cases to isolate a paratyphoid bacillus. Johnston's 4 cases were found among 194, and Hewlett's 1 in a series of 26 cases of typhoid fever. Hünermann has reported an epidemic of 38 cases of paratyphoid infection occurring in the garrison at Saarbrück. Falcioni reports 5 cases out of 100 cases of supposed typhoid fever. The proportion of negative Widal reactions is low in the statistics, but there is a source of error here in that until very recently the tests have not been made in high enough dilutions—that is, at least as high as 1:40.

Post-mortem Findings.—Autopsies were performed on 3 fatal cases (Strong, Longcope, Tuttle). The interest in these autopsies naturally centres on the condition of the intestines. Strong states that both the large and the small intestines were normal throughout except for moderate catarrh and a few superficial hemorrhages. The solitary and agminated follicles showed no lesions. The mesenteric lymphatics, however, and some along the small intestines, were hemorrhagic. In Longcope's case the intestines showed no changes either on gross or microscopic examination. The spleen in both cases was enlarged. The other pathological changes were those common to febrile conditions. In Tuttle's case a few erosions just above the ileocæcal valve were present.

Source of Infecting Bacilli.—Tuttle's case happened to be a laboratory employé in the service of the Department of Health and was carefully investigated by us. We found that two families consisting of eleven members drank water from an open uncovered tank. During the summer the tank was not cleaned and was only occasionally filled by pumping in water from the city supply. Sometimes the water was the color of tea. During a single week four members of one family and three of the other were stricken with a typhoid-like fever. The two families had no social intercourse with each other.

Symptomatology.—It is a significant fact that many of the reported cases of paratyphoid infection were considered to be genuine typhoid fever without the Gruber-Widal reaction until a bacteriological study revealed their character. Tuttle's case had severe hemorrhages and was considered in the hospital as true typhoid infection until the cultures proved it to be paratyphoid. The average course, lasting frequently only 12 to 18 days, is milder. The cases due to the paracolon bacilli are apt to run a course more like those due to the *Bacillus enteritidis* in meat poisoning.

The Serum Reaction in Cases of Paratyphoid Infection.—Since the introduction of serum reactions as a means of diagnosis, it has been a well-recognized fact that a small proportion of cases which are clinically typhoid fever fail to give the reaction. Brill, adding to Cabot's statistics, finds that of 4879 cases 4781, or 97.9 per cent., gave the reaction. Gwyn gives 99.6 per cent. as the percentage of positive reactions in the Johns Hopkins Hospital. On the other hand, in most of the reported cases of paratyphoid infection a reaction, except with low dilutions, against the *Bacillus typhosus* has been absent. It is probable, then, that some at least of the typhoid cases with negative reactions were really paratyphoid infection.

Still it cannot be assumed that all cases clinically typhoid fever, which have been reported as giving the Gruber-Widal reaction, were cases of genuine typhoid infection. The brilliant work of Durham on the typhoid-colon group of bacilli and its serum reactions has brought out the fact that certain members of this group may be mutually interacted upon by sera of infected patients and of immunized animals. This is especially true of sera in low dilution. Since in the earlier

years of the Gruber-Widal reaction the technique had not been worked out, and dilutions were more frequently low than not, some of the cases reported as typhoid fever may have been infections with paratyphoid bacilli.

Diagnosis.—The only reliable criteria for diagnosis are absence of the Gruber-Widal reaction in proper dilution (not less than 1:40) with a positive reaction against a known paratyphoid bacillus or the recovery of a paratyphoid bacillus from the blood, urine, or complicating inflammatory process.

The clinical type of the disease is of little value in a single case. It has already been stated that the reported cases of paratyphoid infection have been both mild and severe.

The cases of paratyphoid infection are too few to state what the *prognosis* should be. It can only be said that the majority of the cases have been mild, though there have been about 9 per cent. of deaths among the cases reported. The differential diagnosis between infections due to the typhoid bacillus and to those due to the paratyphoids and more rarely the paracolons is of importance mainly from the etiological side. If a specific serum therapy is ever successfully instituted the differentiation would be of more importance.

Epidemic Meat-poisoning Type.—Gärtner announced his discovery of *Bacillus enteritidis* as the cause of epidemic meat poisoning in 1888. A cow sick for two days with profuse diarrhœa had been slaughtered in Saxony and the meat sold for food. Of the persons who ate of the meat 57 became ill, and 1 died. Gärtner recovered the bacillus from the meat and from the organs in the fatal case.

Previous to Gärtner's discovery the cause of meat poisoning had been held to be bacterial products, and while this is true of certain instances it is the exception. All cases in which a thorough bacteriological examination has not been made must be excluded.

Two kinds of bacilli are concerned in the production of meat poisoning: 1. *Bacillus enteritidis* of Gärtner, including the different strains of this organism. 2. Anaërobic *Bacillus botulinus* of Van Erminghem, a saprophyte (see later under anaërobic bacilli).

True Meat Poisoning.—This form of meat poisoning is due to *Bacillus enteritidis*, and in every instance the animal is diseased at the time of the slaughter. It may be contracted by eating sausage, since the meat of diseased animals is sometimes surreptitiously put on the market in the form of sausage.

Durham makes *Bacillus enteritidis* the chief type of the intermediates and proposes the name "the enteritidis group." Buxton classes the bacillus with the paracolons. It does not ferment lactose; milk becomes more alkaline; it ferments dextrose with a production of gas containing about one-third CO_2, two-thirds H, and it also ferments mannite, maltose, and dextrin.

Bacillus enteritidis is pathogenic for cows, horses, pigs, goats, mice, and guinea-pigs, but not for dogs and cats.

The Infected Meat.—In many epidemics *Bacillus enteritidis* has

been isolated not only from the organs of fatal cases, but from the suspected meat. The meat does not differ in appearance or taste from that of healthy animals. It has already been stated that it may be made into sausages, and one epidemic at least has been caused by eating "dried meat" consisting of large pieces of the flesh of sheep and goats nearly dried in the sun and eaten cooked or merely softened by soaking. Cooking does not always destroy the bacilli, as the thermal death point may not be reached in the interior of the meat. Infected meat which is not eaten immediately after it has been cooked is especially dangerous.

The meat has always come from animals sick at the time of slaughter. The meat of cows and calves have most often caused the epidemics, though that of horses, pigs, and goats have also been responsible. Durham says that no known case has come from mutton, and that the pig has been implicated in only one outbreak which has been studied bacteriologically. In this connection it is interesting to recall that Theobald Smith has insisted on the similarity between the hog-cholera bacillus and *Bacillus enteritidis*.

The animals from which the infected meat has come have suffered during life from puerperal fever and uterine inflammations, navel infection in calves, septicæmia, septicopyæmia, diarrhœa, and local suppurations, and have not infrequently been killed because of their unsound condition. How animals become infected is not known.

Durham thinks milk may be a source of infection in man, but states that bacteriological evidence of it is incomplete. *Bacillus enteritidis* has been found, however, in the milk of markedly infected guinea-pigs.

Transmission to Man.—The disease may be transmitted to man in two ways: (1) by eating the infected meat, and this is by far the most common means, and (2) from man to man according to Gärtner, Van Erminghem, and Fischer. Fischer thinks transmission may take place through the excreta. *B. psittacosis* has also been transmitted to man.

Epidemics of meat poisoning may occur in any season, but are more frequent during the warm months.

Symptomatology.—While the characteristic symptoms of sausage poisoning relate to the nervous system, in true meat poisoning they are gastrointestinal. Fischer divides meat poisoning into three clinical forms: (1) typhoidal; (2) choleraic; (3) gastroenteric.

Prevention.—Since neither appearance nor taste affords any clue to the noxious quality of the infected meat, its unfitness for food can only be told through bacteriological examination or a knowledge of its source. Thorough cooking will kill the bacilli, but it must be remembered that in this process the thermal death point of the bacilli may not be reached in the innermost portions of the meat.

BACILLUS ALCALIGENES.

This bacillus resembles somewhat a colon bacillus which has lost its power to ferment sugars. Morphologically and culturally it is

more like the typhoid bacillus. It ferments no sugars. It is frequently present in the intestines and may have pathogenic properties, which facts have already been mentioned in speaking of the intermediate group of bacilli (see table p. 257).

BACILLUS OF HOG CHOLERA (B. CHOLERAE-SUIS).

This is an actively motile bacillus. Grows vigorously in bouillon. Renders milk at first slightly acid then strongly alkaline, and dissolves casein. Ferments dextrose with gas production (see table p. 256).

This bacillus is found almost regularly present in hogs sick with cholera, but is known now not to be the essential exciting factor, since this is a virus which passes through a fine filter. Even though now considered not to be the essential factor in exciting hog cholera it is believed to be of importance as an added infection. It is pathogenic for hogs causing, when fed, fatal enteritis.

BACILLUS OF SWINE PLAGUE.

This, a non-motile bacillus which grows feebly in bouillon, does not coagulate milk, and ferments glucose without production of gas. When fed to pigs it does not usually cause illness (see table p. 256). This bacillus is closely related to the hemorrhagic septicemia group.

CHAPTER XXI.

THE DYSENTERY BACILLUS—THE PARADYSENTERY BACILLI (MANNITE FERMENTING TYPES).

DYSENTERY may be divided into acute and chronic. Amœbæ appear to be the chief exciting factor in most cases of chronic dysentery, though bacilli of the colon group also play a part.

In temperate climates acute dysentery is but very rarely due to amœbæ, but usually to the bacilli identified by Shiga or to allied strains identified by Kruse, Flexner, and Park. The usual summer diarrhœas are not excited by the dysentery bacilli.

Historical Note.—In 1897 Shiga found in the stools of cases of dysentery a bacillus which had not been before identified. This bacillus had many of the characteristics of the colon bacillus, but differed from it, lacking motility and failing to produce gas from the fermentation of sugar. It also was entirely distinct in its agglutination characteristics and in its pathogenic properties. Shiga found this bacillus present in all the cases of epidemic dysentery that he examined. It was most abundant during the height of the disease and disappeared with the return of fæcal stools. It was not found in the stools of healthy persons. He found that the blood of dysenteric patients contained substances which agglutinated the bacilli that he had isolated. The serum from healthy individuals did not agglutinate the bacilli to any such degree as the serum from those sick with dysentery. When the mucous membrane of the colon was examined in fatal cases dying in the height of the disease, the bacilli were found in the superficial layers in almost pure cultures. A criminal fed with a culture of the bacillus developed typical dysentery. Certain animals, such as dogs, when subjected to treatment which made them more susceptible, were attacked with dysentery after feeding on cultures. This was fairly similar to that in man.

Morphological and Cultural Characteristics of Dysentery Bacilli.—**Microscopic.**—Similar to bacilli of the colon group.

Staining.—Similar to bacilli of the colon group.

Motility.—No definite motility has been observed. The molecular movement is very active.

Flagella.—True flagella have not been observed by most examiners. On a very few bacilli in suitable smears Goodwin demonstrated what appeared to be terminal flagella. **Spores** are not formed.

Appearance of Cultures.—On *gelatin* the colonies appear more like the typhoid than the colon bacilli. Gelatin is not liquefied. On agar, growth is somewhat more delicate than that of the average colon cultures.

On Potato.—A delicate growth just visible or distinctly brownish.

In Bouillon.—Diffuse cloudiness with slight deposit and sometimes a pellicle. Indol not produced or in a trace only.

In glucose bouillon no production of acid or gas.

Neutral red agar is not blanched.

In Litmus Milk.—After twenty-four to forty-eight hours this becomes a pale lilac. Later, three to eight days, there is a return to the original pale blue color. The milk is not otherwise altered in appearance.

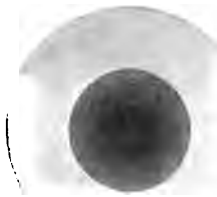
Pathogenesis.—Animal Tests.—No characteristic lesions have followed swallowing large quantities of bacilli. Dogs at times have had diarrhœa with slimy stools, but section showed merely a hyperæmia of the small intestine.

Many animals are very sensitive to bacilli injected into vein or peritoneum; 0.1 mg. of agar culture injected intravenously produced

FIG. 97

Dysentery bacilli. ×1000 diam.

FIG. 98



Colony of dysentery bacilli in gelatin. × 40 diam.

diarrhœa, paralysis, and death; 0.2 mg. under the skin have killed, and the same amount in the peritoneum has caused bloody peritonitis, with lowered temperature and diarrhœa. Both small and large animals are very sensitive to killed cultures.

The autopsy of animals dying quickly from injection into the peritoneum of living or dead bacilli shows the peritoneum to be hyperæmic, the cavity more or less filled with serous or bloody serous exudate. The liver is frequently covered with fibrinous masses. The spleen is moderately or not at all swollen. The small intestine is filled with fluid, the large intestine is usually empty. The mucous membrane of both is hyperæmic and sometimes contains hemorrhages. Conradi found ulcer formation in one case.

Subcutaneous injections of dead or living cultures are followed by infiltration of tissues and frequently by abscess formation. The dysentery bacilli produce both extracellular and cellular toxins, the

latter being the most abundant. The elimination of these toxins from the body is supposed to take place through the intestines, and this gives rise to the intestinal lesions in animals injected intravenously or intraperitoneally. The dysentery bacilli are not found in the blood or organs of animals.

In Man.—In the onset acute dysentery is sudden and ushered in by cramps, diarrhœa, and tenesmus. The stools, at first feculent, then seromucous, become bloody or composed of coffee-ground sediment. At the height of the disease there are ten to fifty stools in the twenty-four hours. After two to seven days the blood usually disappears. In temperate climates the mortality varies from 5 to 20 per cent. Bacillary dysentery is a disease especially of the mucous membrane of the large intestines. The epithelium is chiefly involved. In the lightest cases a catarrhal inflammation is alone present, in the more severe the lymph follicles are swollen and some necrosis of epithelium takes place.

In severe cases in adults the lesions are of a diphtheritic character and may be very marked. The entire lumen of the intestines may be filled with a fibrinous mass of pseudomembrane. In young children, even in fatal cases, the lesions may be more superficial. The following macroscopic and microscopic report of the intestinal findings on a fatal case of bacillary dysentery in an infant is a typical picture:

Small Intestines.—Slightly distended. Mesenteric glands large and red. Peyer's patches are swollen slightly without ulceration.

Large Intestines.—Outer surface vessels congested and prominent, on section, covered with a yellowish mucus. Mucous membrane seems to be absent in places. Solitary follicles are elevated and enlarged, especially in the region of sigmoid flexure. In some instances the centres of the follicles are depressed and appear to be necrotic.

Large Intestine.—Mucous glands are for the most part normal, but over the solitary follicles they have broken down somewhat and contain polynuclear leukocytes. The interglandular stroma in these places has undergone necrosis. The necrotic area extends down into the submucosa in the region of the solitary follicles. The capillaries of the solitary follicles are much dilated and congested. The submucosa is thickened and slightly œdematous. The connective-tissue cells appear to have undergone a slight hyaline degeneration. The musculature is not affected, neither is the peritoneal coat.

Small Intestines.—Normal.

Paradysentery Bacilli as Exciters of Dysentery.—In 1900 Flexner and Strong, when in the Philippine Islands, isolated bacilli from dysenteric stools which were identical with the Shiga cultures. At first all cultures were supposed to be of the Shiga type, but later, among those isolated, bacilli were found, which differed from Shiga's in many characteristics. In the same year Kruse, in Germany, obtained from dysenteric cases in an asylum bacilli which appeared to him to be culturally like those isolated by Shiga, but to differ in their agglutinating characteristics. These, like those isolated by Flexner, were later found to differ in many characteristics. In 1902 Duval and

Bassett, in Baltimore, thought they had found the Shiga bacilli in the stools of a number of cases of summer diarrhœa. These later proved to be identical with some of the bacilli isolated by Flexner in Manila. During the same summer Park and Dunham isolated a bacillus from a severe case of dysentery occurring during an epidemic at Seal Harbor, Mt. Desert, Maine, which they showed to differ from the Shiga bacillus in that it produced indol in peptone solution and differed in agglutinating characteristics.[1] They at first considered it identical with the Philippine culture given them by Flexner, but in January, 1903, it was shown by Park to be a distinct variety, and later found by him to be the exciting factor in a large number of cases in several widely separated epidemics.

Martini and Lentz[2] published the results of their work in December, 1902. They showed that the Shiga type of bacilli obtained from several separate epidemics in Europe agreed with the original Shiga culture in that it did not ferment mannite. The cultures of this type agreed with each other in agglutinating characteristics. When the bacilli from Flexner, Strong, Kruse, Park, Duval, and others, which differed from the Shiga culture in their agglutinins, were tested they were all found to ferment mannite. Martini and Lentz considered that the Shiga bacillus was the true dysentery type and that the mannite-fermenting variety or varieties might be mere saprophytes, or perhaps be a factor in the less characteristic cases.

In January, 1903, Hiss[3] and Russell, independently of others, showed that a bacillus isolated by them from a characteristic stool differed from Shiga's bacillus in the same characteristics as mentioned by Martini and Lentz.

The German observers at first considered the Shiga type as the only one which had established its causal relation to dysentery, while the American observers generally considered both types to have equal standing and some[4] of them considered these differences as not important and perhaps not permanent. This latter opinion seems to have been held by Shiga.[5]

We took up the investigation at this point with the object of carefully studying the bacilli isolated by us from acute dysentery, which occurred in a number of widely separated epidemics. We hoped thus to determine whether the bacilli exciting acute dysentery in the Eastern States belonged to a few distinct types or were divided into a large number of varieties.

In the most extensive epidemic that has recently occurred in the region of New York City there were in all some 500 cases of acute typical dysentery. Whole families were attacked with the disease.

The majority of the cases were of moderate severity, the dysenteric discharges lasting from one to two weeks. There were a number of light cases, but all had dysenteric stools containing mucus and blood.

[1] New York University Bulletin of the Medical Sciences, October, 1902, p. 187.
[2] Zeitschrift f. Hygiene u. Infectionskrank., 1902, xli., 540 and 559.
[3] Medical News, 1903, lxxxii., 289.
[4] University of Pennsylvania Medical Bulletin, July and August, 1903.
[5] Zeitschrift f. Hygiene u. Infectionskrank., 1902, xli., 356.

The mortality was about 6 per cent. Judging from the cases investigated by us, over one-half of those attacked seem to have been infected by the Shiga type, and these were, as a rule, the most severe cases. Most of the cases in two severe, though localized, epidemics in a Pennsylvania town and at Sheepshead Bay were also due to this type. The mortality was higher in these epidemics. The facts published abroad indicate that this variety has been found in the chief epidemics in Europe and Asia. We have never yet succeeded in isolating bacilli which had all the characteristics of the Shiga variety from any diarrhœa cases in which no dysenteric symptoms appeared.

We turn now to the mannite-fermenting varieties, whose relationship to dysentery is still doubted by some.

The cultures isolated by us from over forty cases were found to fall largely into two distinct types, one of which differs from the Shiga bacillus more radically than the other.

The variety nearer to the Shiga bacillus has the characteristics of the culture, which was isolated by us at Seal Harbor, Maine, in August, 1902. The other variety is represented by the Flexner Philippine type.

The first type differs from the Shiga bacillus in its agglutinating characteristics and in that it produces considerable indol in peptone solution and ferments mannite with the production of acids. The second type differs in these points and in addition in its agglutinating characteristics and in fermenting chemically pure maltose in peptone solution.

Besides the epidemic at Seal Harbor, numerous cases of moderately severe or slight dysentery due to the first type were met with in the extensive epidemic which has been already alluded to in the towns north of New York City. A few characteristic and many slightly developed cases of dysentery in New York City during the past two summers were caused by this type of bacillus. A great many cases are also due to the Philippine type. A number of rather severe cases of dysentery developed in Orange, N. J. Cultures from two cases were made, and this latter type alone obtained.

At Riker's Island dysentery broke out in the penitentiary. A considerable number of the inmates including the attendants and doctor in charge came down with the disease. They usually had a short, sharp attack with a quick recovery. Large amounts of blood were passed by some. Those of the infected who were able to work were *sent to the kitchen*. This fact and the facts that open closets were near and that there were immense numbers of flies about were probably responsible for the spread. At the time of the epidemic, a contractor and some workmen were filling in the lower part of the island, about half a mile from the penitentiary. They were not allowed within the penitentiary inclosure. Not one of them contracted the disease.

A large proportion of the bacteria isolated from these dysentery cases were bacilli of the Philippine type. No other type of dysentery bacilli was found in any of the cases in this epidemic.

Charlton and Jehle report a series of cases occurring in Vienna, in which mannite-fermenting types were alone present.

Summer Diarrhœa.—Cases of ordinary enteritis without the symptoms of dysentery are not excited by any of the the types of dysentery bacilli.

Specific or Group Agglutinins Produced by the Three Types.—These are interesting as showing that cultures of each type selected from widely separated sources were identical with each other.

TABLE I.—*Agglutination of bacilli belonging to the three types in the serum of a young goat injected with the bacillus isolated by Shiga, in Japan.*

Source.	Dilutions of Serum. 1:20	1:50	1:100	1:200	1:500	1:2000	1:5000
Type I. Shiga.							
1. Original, Japan—Shiga,	++	++	++	++	++	++	+
2. New Haven—Duval,	++	++	++	++	++	++	+
3. Tuckahoe—Carey,	+|	++	++	++	++	+|	±
4. Coney Island—Collins,	++	++	++	++	++	++	+
5. Mt. Vernon, Case 1.—Collins,	++	++	++	++	++	+|	+
Type II.							
6. Original, Mt. Desert—Park,	+|	+|	|	–	–		
7. New York City—Goodwin,	+|	+|	|	–	–		
8. Hospital, New York—Collins,	+|	+|	|	–	–		
9. Foundling Hospital—Hiss,	+|	+|	|	–	–		
10. Mt. Vernon, Case I.—Collins,	+|	|	–	–	–		
Type III.							
11. Original, Manila—Flexner,	+	+	+	±	–		
12. Baltimore—Duval,	+|	+|	+	±	–		
13. New York City—Wollstein,	++	+	±	|	–		
14. Orange—Collins,	+	+	±	–	–		
15. Riker's Island—Goodwin,	++	++	+	|	–		

The serum of this goat before injection did not agglutinate any of the above bacilli in 1:10 dilution.

++ = complete reaction. + = good reaction. \| = slight reaction.
+\| = very good reaction. ± = fair reaction. – = no reaction.

When the agglutinating characteristics of these bacilli and their susceptibility to immune sera are studied carefully, we find that each of the three types differs from the others. The mannite and the maltose types, since in animals they stimulate abundant common agglutinins and immune bodies, seem more closely allied to each other than to the Shiga type.

This is seen in the accompanying tables, in which bacilli from a number of cases obtained from different sources are tested in sera from animals which have each received a single type of dysentery bacillus:

TABLE II.—*Showing agglutination of members of three types in the serum of animals injected with bacilli of Type II.*

Source.	Goat injected with No. 4. 1:20	1:50	1:100	1:500	1:1000	Rabbit injected with No. 6. 1:20	1:50	1:100	1:500	1:800
Type I. Shiga.										
1. Japan—Shiga,	+	–	–	–	–	|	–	–	–	–
2. New Haven—Duval,	+	–	–	–	–	|	–	–	–	–
3. Tuckahoe—Carey,	+	–	–	–	–	|	–	–	–	–
Type II.										
4. Mt. Desert—Park,	++	++	++	++	++	++	++	++	+	|
5. Mt. Vernon—Collins,	++	++	++	++	++	++	++	++	+	+
6. New York—Hiss,	++	++	++	++	++	++	++	++	+	–
Type III.										
7. Manila—Flexner,	++	++	+	–	–	++	++	++	–	–
8. Baltimore—Duval,	++	++	+	–	–	++	++	++	–	–
9. Riker's—Goodwin,	++	++	+	–	–	++	++	++	–	–

The serum of the above animals previous to immunization did not agglutinate any of the above bacilli in a 1:20 dilution.

TABLE III.—*Showing agglutinations of members of three types in the serum of animals injected with bacilli of Type III.*

	Rabbit injected with Baltimore, Duval.						
	10	50	100	500	1,000	5,000	10,000
Type I.							
1. Japan—Shiga, and 5 other cultures,	++	+	+	−	−	−	−
Type II.							
6. Mt. Desert—Park, and 5 other cultures,	++	++	+	−	−	−	−
Type III.							
Manila—Flexner, and 5 other cultures,	++	++	++	++	++	++	+

Previous to immunization the serum agglutinated the bacilli of Type III. in 1 : 20 dilution but none of the others even in 1 : 10. This is one of the few animals in which agglutinins for Type I. developed through the injections of bacilli of the other types.

TABLE IV.—*Showing how Type III. is unable to absorb the agglutinins produced through injections of Type II. Serum from rabbit inoculated with Mt. Vernon culture, Type II.*

	Serum before absorption.	Agglutinins exhausted with							
		Baltimore, Duval.					Mt. Vernon, cc.²		
		1:20	1:50	1:100	1:200	1:400	1:20	1:50	1:100
Type I.									
Shiga, 5 other cultures,	1:10	−	−	−	−	−	−	−	−
Type II.									
Mt. Desert, 5 other cultures,	1:600	++	++	++	+		−	−	−
Type III.									
Manila, 5 other cultures,	1:100	−	−	−	−	−	++		−

Before injections this rabbit's serum agglutinated Types II. and III. in 1:20 dilutions.

The considerable amount of common agglutinins affecting Type II. and Type III. is seen to be absorbed by the bacilli of either type. The larger amount of specific agglutinin is left in the serum when any bacillus other than one of identical characteristics with the bacillus used in the immunization is employed.

TABLE V.—*Showing that horses injected with Shiga and Philippine types develop specific agglutinins for the bacilli belonging to these two types and common agglutinins for the varieties included under Type II.*

Cultures.	Serum after injections for several months.	Same serum after saturation with cultures of					
		Shiga type.	Type III.	Type II.	Pyocyaneus.	Typhoid.	Colon.
Type I.							
Shiga, original, and 4 others,	+1500	− 10	+400	+700	+1000	+300	+300
Type II.							
Park, original, and 4 others,	+ 600	− 10	− 10	− 10	+ 600	+ 30	+ 50
Type II. (B.)							
Brooklyn	+ 600	+ 20	+ 10	+ 50	+ 300	+100	+ 50
Type II. (C.)	+ 300	− 10	− 10	+ 50	+ 50	+ 10	+ 20
Type II. (D.)	+ 600	− 20	− 10	+ 50	+ 100	+ 30	+ 60
Type III							
Flexner, original, and 4 others,	−1200	+400	− 10	+500	+ 800	+300	+600

The manipulation necessary in making dilutions and filtering, as well as the effect of standing, cause a certain amount of destruction of agglutinins.

Summary.—The great majority of the bacilli which have been isolated from cases of dysentery not due to amœbæ, and which must be considered as being exciting factors in that disease, are included in three distinct varieties of types.

The type most frequently found in severe epidemics is that of the first culture isolated by Shiga. Bacilli identical in biochemical and agglutinating characteristics with this bacillus have been isolated

from cases of dysentery in many parts of the world. None of the bacilli belonging to this type produce indol, except, perhaps, in a trace, or ferment mannite, maltose, or saccharose. Animals injected with this type produce specific agglutinins for this type in abundance and only very little that combines with the others (table I, page 279).

The second type ferments mannite with the production of acid, but does not split maltose or saccharose in peptone solution or agar.

It produces indol. Animals, after inoculations with it, develop immune bodies and agglutinins specific for the type (table II).

The third type is nearest to the colon group, since it not only produces indol and actively ferments mannite, but also acts energetically upon pure maltose and feebly upon saccharose.

These two mannite-fermenting types are widely scattered over the world, and certainly cause characteristic cases and epidemics of dysentery, although on the average the disease caused by them is milder than when due to the Shiga bacillus. One or the other of these two types also appears at times in small numbers in mixed infections where dysenteric symptoms are almost or entirely absent.

Although the majority of bacilli obtained have had the characteristics of one of the above types, a moderate number of bacilli have also been met with which differ slightly in biochemical as well as agglutinating characteristics. Some of these approach very closely the colon bacilli.

It seems, therefore, that these three types should be considered as the characteristic representatives of three groups.

In consideration of all the above facts, it seems to us incorrect to name the mannite-fermenting groups as pseudodysentery bacilli. If we call them all dysentery bacilli, we must classify them as dysentery bacilli of the Shiga group, of the group fermenting mannite, but not maltose, and of the one fermenting both mannite and maltose.

This manner of differentiating the groups would be very confusing, and it seems to us more convenient, and better, to restrict the name dysentery to bacilli having the characteristics of the bacillus isolated by Shiga, and give the name paradysentery to the other two groups which approach more closely to the colon group in that they produce indol and have a greater range of activity in fermenting carbohydrates.

An additional reason for the use of the prefix para, beyond that of convenience, is the less average severity of the disease due to these types, and the probability that there will be found, in occasional sporadic cases and epidemics of dysentery, bacilli which have a causal relation to dysentery and exhibit more pronounced characteristics of the colon group than any of the varieties so far isolated.

Serum Treatment.—In characteristic cases the polyvalent serum is of considerable value. The serum is given subcutaneously in 20 c.c. doses once or twice a day for several days, or until convalescence is established. In cases of the usual summer diarrhœa the serum is not indicated.

CHAPTER XXII.

THE TYPHOID BACILLUS.

THIS organism was first observed by Eberth, and independently by Koch, in 1880, in the spleen and diseased areas of the intestine in typhoid cadavers, but was not obtained in pure culture or its principal biological features described until the researches of Gaffky in 1884. The methods of identification employed by Gaffky were found insufficient to separate the typhoid bacillus from other bacilli of the colon-typhoid group. The absolute identification of the bacillus only became possible with the increase of our knowledge concerning the specific immune substances developed in the bodies of immunized animals. Its etiological relationship to typhoid fever has been particularly difficult of demonstration, for, although pathogenic for many animals when subcutaneously or intravenously inoculated, it has been impossible to produce infection in the natural way or produce gross lesions corresponding closely to those occurring generally in man. Nevertheless the specific reactions of the blood serum of typhoid patients, the constant presence of the *Bacillus typhosus* in the intestines and some of the organs of the typhoid cadavers, the very frequent isolation of this bacillus from the roseola, spleen, blood, and excretions of the sick during life, the absence of the bacilli in healthy persons, unless they have at some time been directly exposed to, or are convalescent from, typhoid infection, all these have demonstrated scientifically that this bacillus is the chief etiological factor in the production of the great majority of cases designated as typhoid fever.

Morphology and Staining.—Typhoid bacilli are short, rather plump rods of about 1μ to 3μ in length by 0.5μ to 0.8μ in diameter, having rounded ends, and often growing into long threads. They are longer and somewhat more slender in form than most of the members of the colon group of bacilli (Figs. 99 and 100).

The typhoid bacilli *stain* with the ordinary aniline colors, but a little less intensely than do most other bacteria. Like the bacilli of the colon and paratyphoid groups, they are decolorized by Gram's method. Bi-polar staining is sometimes marked.

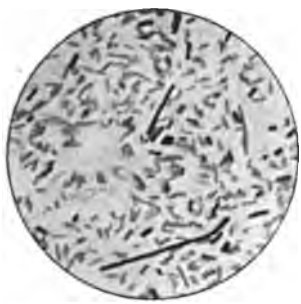
Biology.—The typhoid bacillus is a motile, aërobic, facultative, anaërobic, non-liquefying bacillus. It develops best at 37° C.; above 40° and below 30° growth is retarded; at 20° it is still moderate; below 10° it almost ceases. It grows slightly more abundantly in the presence of oxygen. It does not form spores.

Resistance.—When a number of typhoid bacilli are dried most of them die within a few hours and a few frequently remain alive for months, but sometimes all the bacilli die very quickly. In their

resistance to heat and cold they behave like the average non-spore-bearing bacilli. With rare exceptions they are killed by heating to 60° C. for one minute.

Motility.—Typhoid bacilli, when living under favorable conditions, are very actively motile, the smaller ones having often an undulating motion, while the larger rods move about rapidly. In different cultures, however, the degree of motility varies.

FIG. 99

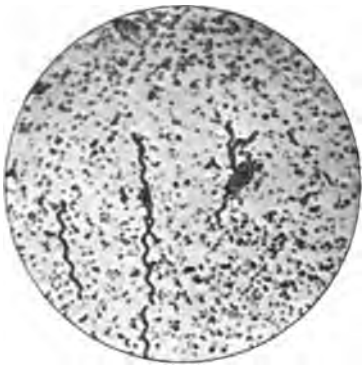


Typhoid bacilli from nutrient agar. ×1100 diameters.

FIG. 100

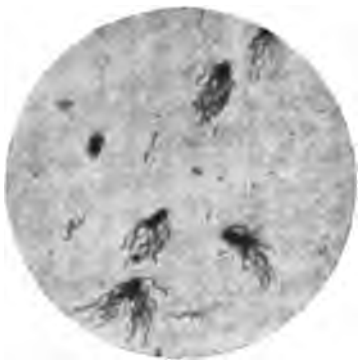
Typhoid bacilli from nutrient gelatin. ×1100 diameters.

FIG. 101



Flagella, heavily stained, attached to bacilli. (Van Ermengen's method.)

FIG. 102



Typhoid bacillus with faintly stained flagella. (Loeffler's method.)

Flagella.—These are often numerous and spring from the sides as well as the ends of the bacilli, but many short rods have but a single terminal flagellum (Figs. 101 and 102).

Cultivation.—Its growth on most sugar-free culture media is quite similar to that of the *Bacillus coli*, but it is somewhat slower and not quite so luxuriant.

Growth on Gelatin Plates (Fig. 103).—The colonies growing deep down in this plate medium have nothing in their appearance to distinguish them from submerged colonies of the colon group; they appear as finely granular round points with a sharp margin and a yellow-

ish-brown color. The superficial colonies, however, particularly when young, are often quite characteristic; they are transparent, bluish-white in color, with an irregular outline, not unlike a grape-leaf in shape. Slightly magnified they appear homogeneous in structure, but marked by a delicate network of furrows. Surface colonies from some varieties of colon bacilli give a similar picture.

In *stick cultures* in gelatin the growth is mostly on the surface, appearing as a thin, scalloped extension, which gradually reaches out to the sides of the tube. In the track of the needle there is but a limited growth, which may be granular or uniform in structure, and of a yellowish-brown color. There is no liquefaction.

FIG. 103

A superficial colony (1) and a deep colony (2) of typhoid bacilli in gelatin. × 20 diameters.

Growth in Bouillon.—This medium is uniformly clouded by the typhoid bacillus, but the clouding is not so intense as by the colon bacillus. When the bouillon is somewhat alkaline a delicate pellicle is sometimes formed on the surface after eighteen to twenty-four hours' growth.

Growth on Agar.—The streak cultures on agar are not distinctive; a transparent, filiform, grayish streak is formed.

Growth on Potato.—The growth on this medium was formerly of great importance in identification, but now other media, giving more specific charactersitics, have been discovered. When characteristic, the growth is almost invisible but luxuriant, usually covering the surface of the medium, and when scraped with the needle offers a certain resistance. In some cases, however, the growth is restricted to the immediate vicinity of the point of inoculation. Again, the growth may be quite heavy and colored yellowish-brown, and with a greenish halo, when it is very similar to the growth of the colon bacillus. These differences of growth on patoto appear to be chiefly due to variations in the substance of the potato, especially in its reaction. For the characteristic growth the potato should be slightly acid. A new lot of potato should always be tested with a typical typhoid bacillus as a control.

Indol Reaction.—It does not, as a rule, produce even a trace indol in peptone-water solution. This test was proposed by Kitasato for differentiating the typhoid bacillus from other similar bacilli such as those of the colon group, which, as a rule, give the indol reaction.

The typhoid bacillus, like the colon bacillus, produces alkaline substances from peptone.

Neutral Red.—In stick cultures in glucose agar the typhoid bacillus produces no change, while the colon bacillus decolorizes the medium and produces gas.

Effect of Inhibiting Substances in Culture Fluids.—The typhoid bacillus is inhibited by weaker solutions of formaldehyde, carbolic acid, and other disinfectants than is the colon bacillus. Most typhoid-like bacilli resemble the typhoid bacillus in this respect. Some substances, such as malachite green, inhibit the colon bacillus more.

Action on Different Sugars.—The determination of the action upon sugars of any bacillus belonging to the typhoid or colon group is one of the most important of all the cultural differential tests. It has been considered in detail in connection with the colon group.

While the typhoid bacillus does not produce gas from dextrose galactose, and levulose, it does produce acid from these substances.

Milk.—The typhoid bacillus does not cause coagulation when grown in milk. In litmus whey the neutral violet color becomes more red during the first forty-eight hours; the fluid, however, remains clear.

Production of Disease in Animals.—It is impossible experimentally to produce the characteristic lesions usually met with in human typhoid fever in animals. Sickness or fatal results without the appearance of the typical pathological changes have regularly followed animal inoculations, but in most cases they could easily be traced to the toxæmia produced by the substances in the bodies of the bacilli injected. Typhoid bacilli, freshly obtained from typhoid cases and introduced subcutaneously in animals, rapidly die. In the peritoneal cavity they may increase, causing a fatal peritonitis with toxic poisoning. By accustoming bacilli to the animal body a certain degree of increased virulence for the animal can be obtained, so that smaller amounts of culture may prove fatal. Among the most successful efforts in this direction are the experiments of Cygnaeus and Seitz, who, by the inoculation of typhoid bacilli into dogs, rabbits, and mice, produced in the small intestines conditions that were histologically, and to the naked eye, analogous to those found in the human subject. Their results, however, were not constant. Very similar results followed inoculation of virulent strains of colon bacilli.

Distribution of Bacilli in the Human Subject. Toxic Effects.—Typhoid fever belongs to that class of infectious diseases in which the specific bacilli are constantly passing into the blood. They thus pass to all parts of the body and become localized in certain tissues, such as the bone marrow, lymphatic tissues and spleen, liver and kidneys. Wherever found in the tissues the typhoid bacilli are usually observed to be arranged in groups or foci; only occasionally are they found singly. These foci are formed, during life, as is proved by the degenerative changes often seen about them; but it is possible that the bacilli may also multiply somewhat after death.

Important Primary Characteristic Lesions in Man.—The lesions of the intestines which are most pronounced in the lower part of the ileum consist of an inflammatory enlargement of the solitary and agminated lymph nodules.

Necrosis with ulceration frequently follows the hyperplasia in the more severe cases. In the severest cases the ulceration and sloughing may involve the muscular and peritoneal coats and perforation may occur. Peritonitis and death usually follow. In rare cases the perforation is closed by adhesions.

The minute changes are an hyperplasia of normal elements of the lymphatic tissue, namely, the lymph cells and the endothelium of the trabeculæ and sinuses. In severer forms necrotic changes are apt to intervene. These changes are attributed to the toxic substances formed by the typhoid bacilli, but may be directly brought about by the occlusion of the nutritive blood vessels, as pointed out by Mallory (Jour. Experimental Medicine, Vol. iii, p. 611).

The mesenteric lymph nodes undergo changes similar to those in the ileum. The spleen is enlarged because of congestion and hyperplasia. The liver and, to a less extent, the kidneys are apt to show foci of cell proliferation.

In typhoid fever, as in other infectious diseases, toxic poisoning may be manifested by disturbances in the circulatory, respiratory, and heat-regulating mechanism as well as by manifest lesions. In a few cases the intestinal lesions are absent. Some of the inflammatory complications which occur in typhoid fever are due to the growth of the bacillus in excessive numbers in unusual places in the body; but many of them are due to a secondary infection with other bacteria, especially the pyogenic cocci and bacilli of the colon group.

Unusual Location of Typhoid Lesions Occurring as Complications of Typhoid Fever.—Cases of sacculated and general peritonitis, abscess of the liver and spleen, subphrenic abscess, osteomyelitis, periostitis, and inflammatory processes of other kinds have been reported as being due to the typhoid bacillus. In certain cases of typhoid pneumonia, serous pleurisy, empyema, and inflammations of the brain and spinal cord or their membranes, typhoid bacilli exclusively have occurred. The inflammation produced may or may not be accompanied by the formation of pus. There are indeed a number of cases now on record in which the typhoid bacillus has played the part of *pus producer.*

Such cases, however, are of comparatively rare occurrence, because only exceptionally do the bacilli mass together in such numbers as to become pus producers.

The Importance of Mixed Infection.—Frequently when complications occur in typhoid fever they are due to secondary or *mixed infection* with the staphylococccus, pneumococcus, streptococcus, pyocyaneus, and colon bacillus. Frequently these bacteria are found side by side with typhoid bacilli; in such cases it is difficult to say which was the primary and which was the secondary infection.

Elimination of Typhoid Bacilli from Body.—Not infrequently typhoid bacilli are found in the secretions. They are present in the urine in about 20 per cent. of the cases in the third and fourth weeks of typhoid fever. Slight pathological lesions in the kidneys almost always occur in typhoid fever, but severe lesions also sometimes occur. In some cases the urine is crowded with typhoid bacilli.

In cases of pneumonia due to the typhoid bacillus it is abundantly present in the sputa, and care should be taken to disinfect the expectoration of typhoid patients. In typhoid fever the bacilli are almost

always present in the gall-bladder. The bacilli are usually eliminated by the fæces, being derived from the ulcerated portions of the intestines; their growth within the intestinal contents is, with few exceptions, not extensive.

Not only do the very great majority of cases examined bacteriologically and pathologically, but the epidemiological history of the disease, proves that the chief mode of invasion of the typhoid bacillus is by way of the mouth and stomach. The infective material is discharged principally by means of the excretions and secretions of the sick—namely, by the fæces, the urine, and occasionally by the sputum.

Occurrence in Healthy Persons.—The bacilli usually disappear from the body in the fourth or fifth week, but may remain for months or exceptionally years in the urine and throughout life in the gall-bladder. They have been found in deep abscesses one year after recovery from typhoid fever.

Typhoid Carriers.—Examinations of convalescent typhoid cases show that about 1 to 5 per cent. continue to pass typhoid bacilli for years, perhaps for life. Petruschky in 1898 reported that typhoid bacilli sometimes remained in the urine of typhoid convalescents for months. Cushing soon after observed a case who had had typhoid fever five years before. In 1902 Frosch, and a little later Conradi and Drigalski, reported persons who passed typhoid-infected fæces months after recovery from typhoid fever. Some bacilli carriers did not know either that they had had typhoid fever or been in contact with it, and others knew only that they had been in contact with it. Lentz in 1905 found out of a large number of examinations that about 4 per cent. of persons convalescent from typhoid fever were typhoid carriers. In our laboratory we have found six in one hundred and forty institution convalescents. The focus of infection is believed to be in either the gall-bladder, chronic ulcers of the intestines, or the normal intestinal tract. The majority are women.

A remarkable case of a cook has been under our care for the past three years. A visitor of the family in which this woman was cook developed typhoid fever some ten days after entering the household. This was in 1901. The cook had been with the family 3 years and it is difficult to judge which infected the other. The cook went to another family. One month later the laundress in this family was taken ill.

In 1902 the cook obtained a new place. Two weeks after arrival the laundress was taken ill with typhoid fever; in a week a second case developed and soon seven members of the household were sick.

In 1904 the cook went to a home in Long Island. There were 4 in the family as well as 7 servants. Within 3 weeks after arrival, 4 servants were attacked.

In 1906 the cook went to another family. Between August 27th and September 3d, 6 out of its 11 inmates were attacked with typhoid. At this time the cook was first suspected. She entered another family on September 21st. On October 5th, the laundress developed typhoid fever.

In 1907 she entered a family in New York City, and two months after her arrival two cases developed, one of which proved fatal. Altogether during five years this cook is known to have been the cause of 26 cases of typhoid fever.

The cook was removed to the hospital March 19, 1907. Cultures taken every few days showed bacilli off and on for three years. Sometimes the stools contained enormous numbers of typhoid bacilli and again for days none would be found. We recently traced some hundreds of cases of typhoid fever to a milk supply produced at a farm, looked after by a typhoid carrier who had typhoid fever forty-seven years ago.

Treatment of Typhoid Carriers.—Medicinal treatment or immunization seems so far to have yielded only slight results. Urotropin in very large amounts is reported to have cured one case, in which operation alone had failed.

Duration of Life Outside of the Body.—It is of importance to know for what length of time the typhoid bacillus is capable of living outside of the body; but, unfortunately, owing to the great difficulties in proving the presence of this organism in natural conditions, our knowledge on this point is still incomplete. In fæces the length of life of the typhoid bacilli is very variable, depending on the composition of the fæces and on the varieties of bacteria present; sometimes they live but a few hours, usually a day, exceptionally for very long periods. Thus, according to Levy and Kayser, in winter typhoid bacilli may remain alive in fæces for five months. Foote says that they can be found in living oysters for a month at a time, but in numerous experiments we have not been able to find them after five days. Their life in privies and in water, however, is usually very much shorter. As a rule, they can be detected in river water no longer than seven days after introduction, and often not after forty-eight hours. The less the general contamination of the water, the longer the bacilli are apt to live. The life of the typhoid bacillus varies according to the abundance and varieties of the bacteria associated with it, and according to the presence or absence of such injurious influences as deleterious chemicals, high temperature, light, desiccation, etc., to which it is known to be sensitive. Good observers claim to have found bacilli very similar to typhoid bacilli in the soil in a region where no typhoid fever was known to exist. The previously mentioned typhoid carriers could account for this. In ice typhoid bacilli rapidly die, none probably ever live as long as six months (see pp. 305–307).

Communicability.—The bacilli may reach the mouth by means of infected fingers or articles of various kinds, or by the ingestion of infected food, milk, water, etc., or by more obscure ways, such as the eating of raw oysters and clams or the contamination of food by flies and other insects, or by inhalation through the mouth. Of the greatest importance, however, is the production of infection by contaminated drinking-water or milk. In a very large number of cases indirect proof of this mode of infection has been afforded by finding

that the water had been contaminated with urine or fæces from a case of typhoid. In a few instances the proof has been direct—namely, by finding typhoid bacilli in the water. Examples of infection from water and milk have frequently come under our direct observation. The following instances may be cited: A large force of workmen obtained their drinking-water from a well near where they were working. Typhoid fever broke out and continued to spread until the well was filled up. Investigation showed that some of the sick, in the early stages of their disease, repeatedly infected the soil surrounding the well with their urine and fæces. Another example occurred in which typhoid fever broke out along the course of a creek after a spring freshet. It was found that, far up near the source of the creek, typhoid fæces had been thrown on one of its banks and had then been washed into the stream.

In the late epidemic at Ithaca some 1500 cases developed among those using the infected water supply of the town. The students and townspeople not drinking the infected supply escaped. The epidemic at Scranton, Pa., during the winter of 1907 was most interesting. A little over 1 per cent. of the inhabitants were attacked. No pollution of the water with typhoid infected fæces or urine could be discovered, although typhoid bacilli were isolated from the water of a small intercepting reservoir by Dr. Fox. This was only accomplished by using large quantities of water. The bacillus isolated was identical by all known tests with the typhoid cultures from cases of typhoid fever.

An instance of milk infection secondary to water infection was in the case of a milk dealer whose son came home suffering from typhoid fever. The fæces were thrown into a small stream which ran into a pond in which the milk cans were washed. A very alarming epidemic of typhoid developed, which was confined to the houses and asylums supplied with this milk. During the Spanish-American war not only water infection, but food infection was noticed, as in the case of a regiment where certain companies were badly infected, while others nearly escaped. Each company had its separate kitchen and food supply, and much of the infection could be traced to the food, the contamination coming partly through the flies. Several epidemics have been traced to oysters.

Individual Susceptibility.—In this, as in all infectious diseases, *individual susceptibility* plays an important rôle in the production of infection. Without a suitable soil upon which to grow, the seed cannot thrive. There must in many be some disturbance of the digestion, excesses in drinking, etc., or a general weakening of the power of resistance of the individual, caused by bad food, exposure to heat, over-exertion, etc., as occurs with soldiers and prisoners, for example, to bring about the conditions suitable for the production of typhoid fever.

The supposition that the breathing of noxious gases predisposes to the disease, though possibly true to a certain extent, as some animal experiments already referred to would seem to indicate, has not yet been conclusively proven; nor do Pettenkofer's investigations into

the relation of the frequency of typhoid fever to the ground-water level satisfactorily explain the occurrence of the disease in most cases, whether sporadically or in epidemics.

Immunization.—After recovery from typhoid fever a considerable immunity is present which lasts for years. This is not absolute, as about 2 per cent. of those having typhoid fever have a second attack, which is usually a mild one. Specific *immunization* against experimental typhoid infection has been produced in animals by the usual method of injecting at first small quantities of the living or dead typhoid bacilli and gradually increasing the dose. The blood serum of animals thus immunized has been found to possess bactericidal and feeble antitoxic properties against the typhoid bacillus. These characteristics have also been observed in the blood serum of persons who are convalescent from typhoid fever. The attempt has been made to employ the typhoid serum for the cure of typhoid fever in man, but, although a number of individual observers have reported good results with one or another of the sera, most consider that little or no good is derived from serum.

Vaccination Against Typhoid.—The use of killed typhoid bacilli as vaccines has been advocated by Wright and tried upon some 8000 persons who expected to be subjected to danger of infection. Two injections are usually given. The first of 500 millions and the second, ten days later, of 750 millions. If it is impossible to count the number 0.1 c.c. and 0.3 c.c. of a bouillon culture can be given. The bacilli are heated to 60° C. for thirty minutes or killed by ½ per cent. lysol or carbolic acid. For a day or two the injection produces a slight fever and local pain, followed in a few days by the development of bactericidal substances in the blood, apparently sufficient in amount to give some immunity lasting for a year or more. A second injection adds to the degree of immunity. In 49,600 individuals under observation in India and Africa, 8600 were thus treated. The disease appeared in them to the extent of 2.25 per cent., with a case mortality of 12 per cent. In the 41,000 uninoculated there was a case percentage of 5.75 per cent., and a case mortality of 26 per cent. The use of protective vaccines in the shape of dead cultures, would, therefore, seem to be advisable where danger of typhoid infection exists.

Vaccination During Typhoid Fever.—The results obtained by Richardson and others do not show any definite effect except that relapses seem to be less.

Diagnosis by Means of the Widal or Serum Reaction.—The chief practical application of our knowledge of the specific substances developed in the blood of persons sick with typhoid fever has been as an aid to diagnosis.

In 1894–95 Pfeiffer showed that when cultures containing dead or living cholera spirilla or typhoid bacilli are injected subcutaneously into animals or man, specific protective substances are formed in the blood of the individuals thus treated. These substances confer

a more or less complete immunity against the invasion of the living germs of the respective diseases. He also described the occurrence of a peculiar phenomenon when some fresh culture of the typhoid bacillus on agar is added to a small quantity of serum from an animal immunized against typhoid bacilli and the mixture injected into the peritoneal cavity of a non-immunized guinea-pig. After this procedure, if from time to time minute drops of the liquid be withdrawn in a capillary tube and examined microscopically, it is found that the bacteria previously motile and vigorous and which remain so in control animals inoculated without the specific serum, rapidly lose their motility and die. They are first immobilized, then they become somewhat swollen and agglomerated into balls or clumps, which gradually become paler and paler, until finally they are dissolved in the peritoneal fluid. This process usually takes place in about twenty minutes, provided a sufficient degree of immunity be present in the animals from which the serum was obtained. The animals injected with the mixture of the serum of immunized animals and typhoid cultures remain unaffected, while control animals treated with a fluid containing only the serum of non-immunized animals mixed with typhoid cultures die. Pfeiffer claimed that the reaction of the serum thus employed is so distinctly specific that it could serve for the differential diagnosis of the cholera vibrion or typhoid bacillus from other vibrions or allied bacilli, such as Finkler's and Prior's or those of the colon group respectively.

In March, 1896, Pfeiffer and Kolle published an article entitled "The Differential Diagnosis of Typhoid Fever by Means of the Serum of Animals Immunized against Typhoid Infection," in which they claimed that by the presence or absence of this reaction in the serum of convalescents from suspected typhoid fever the nature of the disease could be determined. It was further found, if the serum of an animal thoroughly immunized to the typhoid bacillus was diluted with 40 parts of bouillon, and a similar dilution made of the serum of non-immunized animals, and both solutions were then inoculated with a culture of the typhoid bacillus and placed in the incubator at 37° C., that after the expiration of one hour macroscopic differences in the culture could be observed, which increased in distinctness for four hours and then gradually disappeared. The reaction occurring is described as follows: In the tubes in which the typhoid culture is mixed with typhoid serum the bacilli are agglomerated in fine, whitish flakes, which settle to the bottom of the tube, while the supernatant fluid is clear or only slightly cloudy. On the other hand, the tubes containing mixtures of bouillon with cholera or coli serum, or the serum of non-immunized animals, inoculated with the typhoid bacilli, become and remain uniformly and intensely cloudy. These serum mixtures, examined microscopically in a hanging drop, show distinct differences. The typhoid serum mixture inoculated with the typhoid bacilli exhibits the organisms entirely motionless, lying clumped together in heaps; in the other mixtures the bacilli are actively motile.

Similar observations were made independently by Gruber and Durham, who maintained, however, that the reaction described by Pfeiffer was by no means specific, and that when the reaction is positive the diagnosis still remains in doubt, for the reaction is *quantitative* only, and *not qualitative*. They concluded, nevertheless, that these investigations would render valuable assistance in the clinical diagnosis of cholera and typhoid fever.

Gruber-Widal Test.—The first application of the use of serum, however, for the early diagnosis of typhoid fever on a more extensive scale was made by Widal, and reported with great fullness and detail in a communication published in June, 1896. Widal confirmed the reaction as above described, proved that the agglutinative reaction usually occurred early, elaborated the test, and proposed a method by which it could be practically applied for diagnostic purposes. Since then the serum test for the diagnosis of typhoid fever has come into general use in bacteriological laboratories in all parts of the world, and though the extravagant expectations raised at the time when Widal first announced his method of applying this test have not been entirely fulfilled, it has, nevertheless, proved to be of great assistance in the diagnosis of obscure cases of the disease, and is now one of the recognized tests for the differentiation of the typhoid bacillus.

It should also be mentioned that to Wyatt Johnson, of Montreal, belongs the credit of having brought this test more conspicuously before the public, by introducing its use into municipal laboratories, suggesting that dried blood should be employed in place of blood serum (Widal having previously noticed that drying did not destroy the agglutinating properties of typhoid blood); and that in October, 1896, the serum test was regularly introduced in the New York Department of Health Laboratory for the routine examination of the blood serum of suspected cases of typhoid fever. Since then numerous health departments have followed the example set by those of Montreal and New York.

Use of Dried Blood.—Directions for Preparing Specimens of Blood.—The skin covering the tip of the finger or the ear is thoroughly cleansed, and is then pricked with a needle deeply enough to cause several drops of blood to exude. Two fair-sized drops are then placed on a glass slide, one near either end, and allowed to dry. Glazed paper may also be employed, but it is not as good, for the blood soaks more or less into it, and later, when it is dissolved, some of the paper fibre is apt to be rubbed off with it. The slide is placed in a box for protection.

Preparation of Specimen of Blood for Examination.—In preparing the specimens for examination the dried blood, if accuracy is desired, is first weighed and then brought into solution by adding to it the quantity of normal salt solution to make the desired dilution, remembering of course to allow for the loss in water through drying; then a minute drop of this decidedly reddish mixture is placed on a cover-glass, and

to it is added a similar drop of an eighteen to twenty-four-hour-old bouillon culture of the typhoid bacillus, which, if it has a slight pellicle, should be well shaken. The drops, after being mixed, have in a 1:10 dilution a distinct reddish color and in 1:20 a faint pink tinge. The cover-glass with the mixture on the surface is inverted over a hollow slide (the edges about the concavity having been carefully smeared with vaselin, so as to make a closed chamber), and the hanging drop then examined under the microscope by either daylight or artificial light, a high-power dry lens being used, or, somewhat less serviceably, a $\frac{1}{12}$ oil-immersion lens. Ordinarily the dried blood is not weighed, but the measure of dilution is estimated by the color of the drop. To judge this the beginner must carefully make dilutions of fluid blood and notice the depth of color in 1:10 and 1:20 dilutions. Besides the faulty judgment of the dilution color by the examiner, the variation in depth of color of different specimens of blood makes the estimation of dilutions more or less inaccurate, but fortunately this does not greatly interfere with the value of the test

The Reaction.—If the reaction takes place rapidly the first glance through the microscope reveals the reaction almost completed, most of the bacilli being in loose clumps and nearly or altogether motionless (Fig. 104). Between the clumps are clear spaces containing few or no isolated bacilli. If the reaction is a little less complete a few bacilli may be found moving slowly between the clumps in an aimless way, while others attached to the clumps by one end are apparently trying to pull away, much as a fly caught on fly-paper struggles for freedom. If the agglutinating substances are present, but still less abundant, the reaction may be watched through the whole course of its development. Immediately after mixing the blood and the culture together it will be noticed that the bacilli move more slowly than before the addition of serum. Some of these soon cease all progressive movement, and it will be seen that they are gathering together in small groups of two or more, the individual bacilli being still somewhat separated from each other. Gradually they close up the spaces between them, and clumps are formed. According to the completeness of the reaction, either all of the bacilli may finally become clumped and immobilized or only a small portion of them, the rest remaining freely motile, and those clumped may appear to be struggling for freedom. With blood containing a large

FIG. 104

Gruber-Widal reaction. Bacilli gathered into one large and two small clumps, the few isolated bacteria being motionless or almost so.

amount of agglutinating substances all the gradations in the intensity of the reaction may be observed, from those shown in a marked and immediate reaction to those appearing in a late and indefinite one, by simply varying the proportion of blood added to the culture fluid.

Pseudoreactions.—If too concentrated a solution of dried blood from a healthy person is employed a picture is often obtained which may be mistaken for a reaction. Dissolved blood always shows a varying amount of detritus, partly in the form of fibrinous clumps, and prolonged microscopical examination of the mixture of dissolved blood with a culture fluid shows that the bacilli, inhibited by substances in the blood, often become more or less entangled in these clumps, and in the course of one-half to one hour very few isolated motile bacteria are seen. The fibrinous clumps alone, especially if examined with a poor light by a beginner, may be easily mistaken for clumps of bacilli. Again, the bacilli may become fixed after remaining for one-half to two hours, by slight drying of the drop or the effect of substances on the cover-glass. The reaction in typhoid is chiefly due to specific substances, but clumping and inhibition of movement similar in character may be caused by other substances such as exist in normal horse and other serums. This is a very important fact to keep in mind. (For details of technique see pages 42–46.)

In pseudoreactions Wilson has noticed that many free bacilli are apt to be gathered at the margin of the hanging drop.

Mode of Obtaining Serum from Blood or Blisters for Examination.—Fluid blood serum can easily be obtained in two ways: First, the serum may be obtained *directly from the blood*, thus: The tip of the finger or ear is pricked with a lancet-shaped needle, and the blood as it issues is allowed to fill by gravity a capillary tube having a central bulb. The ends of the tube are then sealed by heat or melted wax, or candle-grease, and as the blood clots a few drops of serum separate. To obtain larger amounts of serum for a microscopic examination the blood is milked out from the puncture into a small homœopathic vial or test-tube. One cubic centimetre of blood can easily be collected in this way. The vial is then corked and placed on the ice to allow the serum to separate. As a rule, one or two drops of serum are obtainable at the end of three or four hours. Second, the serum may be obtained *from blisters*. This gives more serum, but causes more or less delay. The method is as follows: A section of cantharides plaster, the size of a 5-cent piece, is applied to the skin at some spot on the chest or abdomen. A blister forms in from six to eighteen hours. This should be protected from injury by a vaccine shield or bunion plaster. The serum from the blister is collected in a capillary tube, the ends of which are then sealed. Several drops of the serum can easily be obtained from a blister so small that it is practically painless and harmless. The serum obtained is clear and admirably suited for the test. A piece of blotting-paper soaked in strong ammonia when placed on the skin and covered by a watch-

glass or strips of adhesive plaster will quickly raise a blister. A little vaselin should be smeared on the skin surrounding the blotting-paper.

Advantages and Disadvantages of Serum, Dried Blood, and Fluid Blood for the Serum Test.—The dried blood is easily and quickly obtained, and does not deteriorate or become contaminated by bacterial growth. It is readily transported, and seems to be of nearly equal strength with the serum in its agglutinating properties. It must in use, however, be diluted with at least five times its bulk of water, otherwise it is too viscid to be properly employed. The amount of dilution can only be determined roughly by the color of the resulting mixture, for it is impossible to estimate accurately the amount of dried blood from the size of the drop, and it is generally considered too much trouble to weigh it accurately. Serum, on the other hand, can be used in any dilution desired, varying from a mixture which contains equal parts of serum and broth culture to that containing 1 part of serum to 100 parts of culture or more, and this can be exactly measured by a graduated pipette or, roughly, by a measured platinum loop. The disadvantages in the use of serum are entirely due to the slight difficulty in collecting and transporting it, and the delay in obtaining it when a blister is employed. If the serum is obtained from blood after clotting has occurred a greater quantity of blood must be drawn than is necessary when the dried-blood method is used; if it is obtained from a blister, a delay of one to eighteen hours is required. The transportation of the serum in capillary tubes presents no difficulties if tubes of sufficiently thick and tough glass are employed and placed in tiny wooden boxes. For scientific investigations and for accurate results, particularly in obscure cases, the use of fluid serum is to be preferred to dried blood. Practically, however, the results are nearly as good for diagnostic purposes from the dried blood as from the serum.

Fluid Blood.—When properly obtained this gives good results. The Thoma-Zeiss blood pipette is very useful. Lance finger-tip or ear and draw the blood into the pipette to the mark 0.5. Then *distilled water* is sucked up in sufficient amount to make the desired solution. One loop of this is added to one loop of bouillon culture.

The Culture to be Employed.—It is important that the culture employed for serum tests should be a suitable one, for although all cultures show the reaction, yet some respond much better and in higher dilutions than others. Cultures freshly obtained from typhoid cases are not as sensitive as those grown for some time on nutrient media Those kept for a long time on artificial media sometimes show a decided tendency to spontaneous agglutination. Decrease in virulence is apt to be accompanied by increase of capacity for agglutination. For the past fifteen years we have used a culture obtained from Pfeiffer. A broth culture of the typhoid bacillus developed at 25° to 35° C., not over twenty-four hours old, in which the bacilli are isolated and actively motile, has been found to give us the most satisfactory results. Cultures grown at temperatures over 38° C. are not apt to agglutinate so well as those grown at lower temperatures. Stock cultures of typ-

phoid bacilli can be preserved on nutrient agar in sealed tubes and kept in the ice-box. These remain alive for months or even years. From time to time one of these is taken out and used to start a fresh series of bouillon cultures.

Dilution of the Blood Serum to be Employed and Time Required for the Development of Reaction.—The serum test, as has been pointed out, is quantitative and not qualitative. By this it is not meant to assert that all the agglutinating substances produced in the blood of a patient suffering from typhoid infection are the same as those present in small amount in normal blood, or those produced in the blood of persons sick from other infections. It is true, however, that the apparent effect upon the bacilli of specific and group agglutinins is identical, the difference being that in typhoid fever, as a rule, the specific substances which cause this reaction are usually far in excess of the amount of the non-specific which ever appears in non-typhoid blood, so that the reaction occurs after the addition to the culture of far smaller quantities of serum than in other diseases, or when the same dilution is used it occurs far more quickly and completely with the typhoid serum. (See chapter on agglutinins.) It is most important to remember that it is purely a matter of experience to determine in any type of infection what agglutinating strength of a serum is of diagnostic value.

The results obtained in the Health Department laboratories, as well as elsewhere, have shown that in a certain proportion of cases not typhoid fever a slow reaction occurs in a 1:10 dilution of serum or blood; but very rarely does a complete reaction occur in this dilution within *fifteen minutes*. When dried blood is used the slight tendency of non-typhoid blood in 1:10 dilution to produce agglutination is increased by the presence of the fibrinous clumps, and perhaps by other substances derived from the disintegrated blood cells.

From many cases examined it has been found that in dilutions of 1:20 a quick reaction is almost never produced in any febrile disease other than due to typhoid or paratyphoid bacillus infection, while in typhoid fever such a distinct reaction often occurs with dilutions of 1:100 or more. It is possible that some cases of paratyphoid infection give a prompt reaction in 1:20 dilutions, but if this is so, it is not a serious drawback. The very rare cases of persons who though never having had typhoid fever yet are typhoid bacillus carriers usually have specific agglutinins in their blood.

The mode of procedure, therefore, as now employed is as follows: The test is first made with the typhoid bacillus in a 5 per cent. solution of serum or blood. In the case of serum, one part of a 1:10 dilution is added to one of the bouillon culture. With dried blood, a solution of the blood is first made, and the dilution guessed from the color. To obtain an idea of the dilution by the color, known amounts of blood are dried and then mixed with definite amounts of water; the colors resulting are fixed in the memory as guides for future tests. If there is no reaction—that is to say, if within five minutes no marked change is noted in the motility of the bacilli, and no clumping occurs—nothing

more is needed; the result is negative. If marked clumping and immobilization of the bacilli immediately begin and become complete within five minutes, this is termed a *marked immediate typhoid reaction*, and no further test is considered necessary, though it is always advisable to confirm the reaction with higher dilutions up to 1:50 or more, so as to measure the exact strength of the reaction. If in the 1:20 dilution a complete reaction takes place within thirty minutes, the blood is considered to have come from a case of typhoid infection, while if a less complete reaction occurs it is considered that only a probability of typhoid infection has been established. By many the time allowed for the development of the reaction with the high dilutions is from one to two hours, but to us twenty minutes with the comparatively low dilution of 1:20 seems safer and more convenient. Positive results obtained in this way may be considered conclusive, unless there be grounds for suspecting that the reaction may be due to a previous fairly recent attack. The failure of the reaction in one examination by no means excludes the presence of typhoid infection. If the case clinically remains doubtful, the examination should be repeated every few days.

Use of Dead Cultures.—Properly killed typhoid bacilli respond well to the agglutination test. For the physician at his office the dead bacilli offer many advantages. The reaction is slower than with the living cultures and is observed either macroscopically or microscopically. A number of firms now supply outfits for the serum test. These outfits consist of a number of small tubes containing an emulsion of dead typhoid bacilli. Directions accompany the outfit.

Proportion of Cases of Typhoid Fever in which a Definite Reaction Occurs, and the Time of its Appearance.—As the result of a large number of cases examined in the Health Department Laboratories, it was found that about 20 per cent. give positive results in the first week, about 60 per cent. in the second week, about 80 per cent. in the third week, about 90 per cent. in the fourth week, and about 75 per cent. in the second month of the disease. In 88 per cent. of the cases in which repeated examinations were made (hospital cases) a definite typhoid reaction was present at some time during the illness.

Persistence of the Reaction.—In persons who have recovered from typhoid fever this peculiar property of the blood serum may persist for a number of months. Thus a definite typhoid reaction has been observed from three months to a year after convalescence, and a slight reaction, though much less than sufficient to establish a diagnosis of typhoid infection, from one to fifteen years after the disease. In persons in whom the typhoid bacilli persist the serum reaction may last as long as the bacilli remain in the body.

Reaction with the Blood Serum of Healthy Persons and of Those Ill with Diseases other than Typhoid Fever.—In the blood serum of over one hundred healthy persons examined in the Health Department laboratories an immediate marked reaction has not been observed in a 1:10 dilution. In several hundred cases of diseases, eventually not believed by the physicians in charge to be typhoid fever, only

very rarely did the serum give a marked immediate reaction in a 1:10 dilution. In the light of past experience, I believe a typhoid or paratyphoid infection, though not a typical typhoid fever, to have existed in these cases. These results have been confirmed by others, the question of dilution having recently been made the subject of elaborate investigations, with the view of determining, if possible, at what dilution the typhoid serum would react while others would not. Thus, Schultz reports that among 100 cases of non-typhoid febrile diseases apparently positive results were obtained in 19 with dilutions of 1:5, in 11 of these with 1:10, in 7 with 1:15, in 3 with 1:20, and in 1 a very faint reaction with 1:25; whereas, in as many cases of true typhoid he never failed with dilutions of 1:50. In these experiments it must be noted, however, that the time limit was from one to two hours. A faint reaction with a 1:25 dilution with a time limit of two hours indicates less agglutinating substance than an immediate complete reaction with a 1:10 dilution.

From an experience with the practical application of the serum test for the diagnosis of typhoid fever extending over many years, it may be said that this method of diagnosis is simple and easy of performance in the laboratory by an expert bacteriologist, but it is not to be recommended for routine employment by practising physicians as a clinical test unless they have had experience; that with the modifications as now employed, and due regard to the avoidance of all possible sources of error, it is as reliable a method as any other bacteriological test at present in use; and that as such the Gruber-Widal test is an indispensable, though not absolutely infallible, aid to the clinical diagnosis of irregular or slightly marked typhoid fever.

Isolation of Typhoid Bacilli from Suspected Fæces, Urine, Blood, Water, etc.—In the bacteriological study of typhoid infection for diagnostic and other purposes, attempts have been made to isolate the specific bacilli from the blood, rose spots, sweat, urine, fæces, and by spleen puncture. Although the results obtained by puncture of the spleen have been encouraging and have thrown light upon the distribution of the organism in the body during life, yet as a regular means of diagnosis it is to be discouraged, on account of the possible danger to the patient. The results of the examination of the blood and rose spots of typhoid patients have until recently proved unsatisfactory, investigations of some of the later observers have given a large percentage of positive results from the blood. The examination of the urine and fæces of typhoid patients has often given positive results, and these positive results have become more frequent and satisfactory as the methods for differentiating the *Bacillus typhosus* have grown more exact and refined.

Several media recently devised for the isolation and identification of the typhoid bacillus are much better than any of those formerly used. These are the Hiss, Capaldi, Endo and the Drigalski-Conradi media. In the hands of trained bacteriologists they give satisfactory results.

The Hiss Media: Their Composition and Preparation.[1]—Two media are used: one for the isolation of the typhoid bacillus by plate culture, and one for the differentiation of the typhoid bacillus from all other forms in pure culture in tubes.

The plating medium is composed of 10 grams of agar, 25 grams of gelatin, 5 grams of sodium chloride, 5 grams of Liebig's beef extract, 10 grams of glucose, and 1000 c.c. of water. When the agar is thoroughly melted the gelatin is added and completely dissolved by a few minutes' boiling. The medium is then titrated, to determine its reaction, phenolphthalein being used as the indicator. The requisite amount of normal hydrochloric acid or sodium hydrate solution is added to bring it to the desired reaction—*i. e.*, a reaction indicating 2 per cent. of normal acid. To clear the medium add one or two eggs, well beaten in 25 c.c. of water, boil for forty-five minutes, and filter through a thin filter of absorbent cotton. Add the glucose, after clearing. The reaction of the medium is most important; it should never contain less than 2 per cent. of normal acid.

FIG. 105

Hiss' plate media: Small light colony (*t*) is composed of typhoid bacilli; large colony (*c*) of colon bacilli. (From Hiss.)

The tube medium contains agar, 5 grams; gelatin, 80 grams; sodium chloride, 5 grams; meat extract, 5 grams, and glucose, 10 grams to the litre of water, and reacts 1.5 per cent. acid by the indicator. The mode of preparation is the same as for the plate medium, care being taken always to add the gelatin after the agar is thoroughly melted, so as not to alter this ingredient by prolonged exposure to high temperature. The glucose is added after clearing. The medium must contain 1.5 per cent. of normal acid.

GROWTH OF THE COLONIES.—The growth of the typhoid bacilli *in plates* made from the medium as above described gives rise to small colonies with irregular outgrowth and fringing threads (Fig. 105). The colon colonies, on the other hand, are much larger, and, as a rule, are darker in color and do not form threads. The growth of the typhoid bacilli *in tubes* produces uniform clouding at 37° C. within eighteen hours. The colon cultures do not give the uniform clouding, and present several appearances, probably dependent upon differences in the degree of their motility and gas-producing properties in media. Some of the varieties of the colon bacillus grow only locally where they were inoculated by the platinum needle. Others grow diffusely through the medium, but owing to the production of gas and the passage of gas-bubbles through the medium, clear streaks ramify through the otherwise diffusely cloudy tube contents. This characteristic appearance is not produced when the medium is incorrect in reaction or in consistency. With untried media it is always well to insert a platinum wire into the tube contents and stir them about: if any gas is liberated the culture is not one of the typhoid bacillus and the medium is not correct.

METHOD OF MAKING THE TEST.—The usual method of making the test is to take enough of the specimen of fæces or urine—*i. e.*, from one to several loops—and transfer it to a tube containing broth. From this emulsion in broth five or six plates are generally made by transferring one to five loops

[1] This description is taken from an article by Dr. Philip Hanson Hiss, Jr., "On a Method of Isolating and Identifying Bacillus Typhosus and Members of the Colon Group in Semisolid Culture Media," published in the Journal of Experimental Medicine, 1897, vol. ii., No. 6.

of the emulsion to tubes containing the melted plate medium, and then pouring the contents of these tubes into Petri dishes. These dishes are placed in the incubator at 37° C. and allowed to remain for eighteen to twenty-four hours, when they may be examined. If typical thread-forming colonies are found the tube medium is inoculated from them, and the growth in the tubes allowed to develop for about eighteen hours at 37° C. If these tubes then present the characteristic clouding, experience indicates that the diagnosis of typhoid may be safely made, for the typhoid bacillus alone, of all the organisms investigated, has displayed the power of giving rise both to the thread-forming colonies in the plating medium and the uniform clouding in the tube medium when exposed to a temperature of 37° C. The organisms isolated in this manner have been subjected to the usual tests for the recognition of the *Bacillus typhosus*, and have always corresponded in all their reactions to those given by the typical typhoid bacillus.

The Capaldi Plate Medium.—In his original paper, Capaldi gives the following recipe:

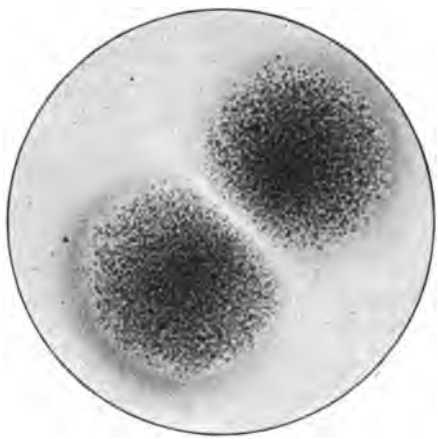
Aqua dest	1000
Witte's peptone	20
Gelatin	10
Mannite or grape-sugar	10
Sodium chloride and potassium chloride each	5

Boil, filter, add 2 per cent. agar and 10 c.c. of normal sodic hydrate solution; boil, filter, and sterilize.

In making up the medium for work the only variation was that in the original recipe the agar was added when the gelatin was put in, now the gelatin is added after the first filtration.

The Capaldi medium is usually employed for surface cultures, but can be inoculated while melted in the tubes. Plates may be made beforehand, so that they are ready for use when the specimen comes in. As these plates are to be kept at 37° C., the difficulties in regard to temperature are avoided; but, unlike the Elsner plates, other organisms besides the colon and typhoid develop and may cause some confusion. In making the plates one or two are inoculated by gently carrying across their surface a platinum loop of fæces or urine. Others are then inoculated with a loop of urine or much diluted fæces. In this way we are apt to have some plates with just the right amount of colonies.

FIG. 106



Colonies of colon bacilli on Capaldi medium slightly magnified. Typhoid colonies of same size usually have no dark granules.

APPEARANCE OF THE COLONIES.—Capaldi thus describes the differentiation: Typhoid—Small, glistening, transparent, almost colorless colonies (by reflected light, blue). Colon—Large, milky colonies (reflected light, brown).

In using the medium it was found that even in a pure plate of typhoid the colonies vary much in size and appearance, while different typhoids show individual differences in growth. In general, a medium-sized, *gray-white* colony, with a few refractive granules, is the typhoid. However, it is often transparent, without the refractive granules; sometimes with a nuclear cen-

tre, and sometimes of equal consistency throughout. Streptococci simulate typhoid, but a high-power lens will show the coccus.

Colon colonies are usually much larger than the typhoid—a decided brown color, very large, refractive granules, and in general quite different in appearance (Fig. 106).

The best way to work with the Capaldi medium is to make several plates with different typhoid cultures, observe carefully all the variations in the colonies, and bear these in mind when working with the mixed plates. After these precautions have been taken the medium will be found very satisfactory. The colonies, as a rule, appear characteristically in twelve to eighteen hours, and thus give a quick method of diagnosis.

We found that the two media (Capaldi and Hiss) work excellently together, as one is an aid to the other. When many colonies of the typhoid bacilli were present the points of differentiation were usually easily seen upon both media, and the two together made diagnosis almost certain. The bacilli from the suspected typhoid colonies can be quickly tested, sufficiently for practical purposes, on the Hiss tube medium, and by the reaction between the bacilli and the serum from an immunized horse.

Typhoid Medium of von Drigalski and Conradi. — These authors modified lactose litmus agar by adding to it nutrose and crystal violet and by using 3 per cent. of agar instead of 2 per cent. The crystal violet strongly inhibits the growth of many other bacteria, especially cocci, which would also color the medium red; the 3 per cent. agar makes the diffusion of the acid which is formed more difficult.

Three pounds of chopped beef are allowed to stand twenty-four hours with 2 litres of water. The meat infusion is boiled one hour and filtered. Twenty grams Witte's peptone, 20 grams nutrose, and 10 grams of salt are then added, and the mixture boiled another hour. After filtration and the addition of 60 grams agar the mixture is boiled for three hours, alkalized and filtered. In the meantime 300 c.c. litmus solution (Kahlbaum) are boiled for fifteen minutes with 30 grams lactose. Both solutions are then mixed and the mixture, which is now red, faintly alkalized with 10 per cent. soda solution. To this feebly alkaline mixture 4 c.c. hot sterile 10 per cent. soda solution are added and 20 c.c. of a sterile solution (0.1 : 100) of crystal violet Höchst B. A substitute for Kahlbaum's litmus solution can be made as follows:

One pound of litmus cubes are ground in mortar to a fine powder and extracted three times with boiling alcohol—500 c.c. each time. This is twice extracted with boiling water—1000 c.c. each time.

The extract is evaporated down to a saturated solution and made acid with hydrochloric acid. It is then placed in a dialyzing bag and dialyzed for six days in running water. It is again evaporated down to a saturated solution and 10 per cent. absolute alcohol added when it is cool.

Enough one one-hundredth normal HCl is added so that one drop *more* brings about a distinct red color.

Plates are inoculated on the surface only. The material to be examined (stools first diluted with ten volumes of 0.8 per cent. salt solution) is spread directly on the surface of the plates, and these inverted are allowed to stand slightly open for about half an hour in order that they may dry somewhat. They are then placed inverted

into the incubator for from sixteen to twenty-four hours. Typhoid colonies are small (1 to 3 mm.), transparent, and blue; colon colonies are red, coarser, less transparent, and larger. The colonies of fresh alkaligenes are blue and usually larger. The suspected colonies can at once be tested for agglutination with a high-grade typhoid serum.

In general this method has withstood critical tests and it is nowadays regarded as perhaps the very best.

As to the comparative merits of the three media, it is probably safe to say that any one of them will, in the hands of one accustomed to them, reveal the typhoid bacilli, except perhaps when they exist in only the most minute numbers. The Hiss plate medium has the objection that it is a difficult medium to prepare. If the acidity is not just right the thread outgrowths do not appear. Indeed, the only sure way is to test a new batch of medium with a pure culture and alter the reaction until the culture grows correctly. A very few strains of the typhoid bacillus do not produce typical thread outgrowths from the colonies. In the Drigalski medium the typhoid colonies are easily separated from those of the colon bacilli, but there are other intestinal bacteria which grow fairly like them.

The Capaldi medium has the objection that some of the typhoid and some of the colon colonies frequently look much alike. If one, however, will always pick out the colonies which look most like the typhoid, it will usually turn out that typhoid bacilli have been obtained if any were present. Personally, for general use, I prefer the Drigalski medium for the plate cultures and the Hiss tube medium for the first step in identifying the bacilli obtained. Through these media and specific agglutinating serum we are now in a position to obtain and make a fairly accurate identification of typhoid bacilli from fæces, urine, etc., within forty-eight hours.

Endo Medium for Typhoid Differentiation.[1]—Fuchsin solution prepared by adding 10 grams fuchsin (not acid) to 100 c.c. 96 per cent. alcohol. Shake and allow to stand for twenty hours, decant and filter supernatant fluid. Always filter before using.

Make 4 per cent. nutrient agar as follows: 1 liter water, 5 grams sodium chloride, 10 grams Liebig's meat extract, 10 grams peptone; dissolve by heating, cool and add 40 grams agar; cook in Arnold three hours and then filter through cotton or perforated funnel (Buchner) by aid of vacuum, neutralize to litmus-paper with Na_2CO_3 solution and add 10 c.c. sterilized 10 per cent. Na_2CO_3 solution to alkalinity; add 10 grams C. P. lactose (important to have C. P.); add 5 c.c. of above alcoholic fuchsin solution; add 50 c.c. freshly made and sterilized 10 per cent. sodium sulphite solution; tube and sterilize for a short time in Arnold.

The medium after cooling should be nearly colorless to transmitted light and rose- or flesh-colored to reflected light. The lactose

[1] Endo, *Centblt. f. Bakt.*, 35, 1904, p. 109. Klinger, Arb. a. d. Kais. Ges., 1906, p. 52. Willson, *J. of Hyg.*, 1905, p. 429. Kayser, *Munch. med. Woch.*, 1906, pp. 17–18.

fuchsin, and sodium sulphite solutions must be added to the melted agar just before it will be used. The plates are poured and allowed to stand twenty minutes uncovered in the incubator in order to do away with water of condensation and to obtain a good surface. The plates should be neither too moist nor too dry.

Organisms which split lactose restore the red fuchsin and appear as deep red sharply limited opaque colonies with a greenish surface shown.

The typhoid organism produces smaller transparent colonies resembling a small drop of water.

Typhoid Bacilli in Fæces.—Recently numerous investigations have been carried out to discover how frequently and at what period in typhoid fever bacilli are present in the fæces and urine. Hiss some time ago examined the fæces of 43 consecutive cases, 37 of which were in the febrile stage and 6 convalescent. In a number of instances only one stool was examined, but even under these adverse conditions the average of positive results in the febrile stage was 66.6 per cent. Out of 26 cases of typhoid fever examined in hospitals, 21 were in the febrile stage and 5 convalescent. In the febrile cases in 17 the presence of typhoid bacilli, often in great numbers, was demonstrated. Thus in these carefully followed cases the statistics show over 80 per cent. of the febrile cases positive. The bacilli were isolated from these cases as early as the sixth day, and as late as the thirtieth day, and in a case of relapse on the forty-seventh day of the disease. The convalescent cases examined gave uniformly negative results, the earliest examination having been made on the third day after the disappearance of the fever. The bacilli seemed to be more numerous in the stools from about the tenth or twelfth day on. These observations, with regard to the appearance of the bacilli in the stools during the febrile stage and their usually quick disappearance, except in the permanent typhoid carriers, after the defervescence, have been confirmed by others. The bacilli were isolated in several cases in which no Widal reaction was demonstrated. Between the seventh and twenty-first days of the disease, experience seems to indicate that the bacilli may be obtained from about 25 per cent. of all cases on the first examination and from about 75 per cent. after repeated examinations. In some samples of fæces typhoid bacilli die out within twenty-four hours; in others they remain alive for days or even weeks. This seems to depend on the bacteria present in the fæces and upon its chemical character. Probably the presence of typhoid bacilli in some stools and their absence in others must be explained largely by the characteristics of the intestinal contents. The short life of the typhoid bacillus in many specimens of fæces suggests that stools be examined as quickly as possible. In fact, unless the physician wishes to take the trouble to have the sample of fæces sent immediately to the laboratory, it is hardly worth while for the bacteriologist to take the trouble to make the test.

Typhoid Bacilli in the Urine.—Of great interest is the frequent

occurrence of typhoid bacilli in large numbers in the urine. The results of the examinations of others as well as our own indicate that the typhoid bacilli are not apt to be found in the urine until the beginning of the third week of the fever, and may not appear until much later. From this on to convalescence they appear in about 25 per cent. of the cases, usually in pure culture and in enormous numbers. Of 9 positive cases examined by Richardson[1] 2 died and 7 were discharged. At the time of their discharge their urine was loaded with typhoid bacilli. We have observed similar cases. In one the bacilli persisted for five weeks. Undoubtedly in exceptional cases they persist for years. When we think of the chances such cases have to spread infection as they pass from place to place, we begin to realize how epidemics can start without apparent cause. The more we investigate the persistence of bacteria in convalescent cases of disease, the more difficult the prevention of their dissemination is seen to be. The disinfection of the urine should always be looked after in typhoid fever, and convalescents should not be allowed to go to places where contamination of the water supply is possible, without at least warning them of the necessity of great care in disinfecting their urine and fæces for some weeks. Richardson made the interesting discovery that after washing out the bladder with a very weak solution of bichloride of mercury the typhoid bacilli no longer appeared in the urine.

Typhoid Bacilli in Blood.—In many cases typhoid bacilli are found in small numbers in the blood *early in the course of the disease.* They continue to be present until the height of the fever, when they decrease, owing to the increase of bactericidal substances. Thus the early bacteriological examination of the blood may be an important aid in early diagnosis.

The following methods are recommended for this blood examination: (1) *Schottmuller's method:* 1.5–2 c.c. of blood grown in 100 c.c. of nutrient broth. (2) *Conradi's bile-enriching method:* 2–5 c.c. of blood grown in 10 c.c. bile mixture (beef bile + 10 per cent. peptone + 10 per cent. glycerin). (3) *Meyerstein's*[2] *enriching method with concentrated bile salts:* 2–3 c.c. of blood are well shaken with 2–3 drops of bile salts solution (20–40 per cent. of pulverized bile-acid salts in equal parts of glycerin and distilled water). (4) *Rosen-Runge's method:* 1 per cent. sodium glycocholate added to nutrient agar. In each tube containing 10–15 c.c. of this medium melted, 2 c.c. of blood is added and plates are poured.

Of these four methods, Meyerstein's was found by Bohne[3] to be the most satisfactory.

Bile media are supposed to allow a good growth of typhoid bacilli and at the same time to inhibit the growth of possible contaminations.

Detection of Typhoid Bacilli in Water.—There is absolutely no

[1] Journal of Experimental Medicine, May, 1898.

[2] Meyerstein, W. Ueber Typhusanreicherung. Münch. med. Woch., 1906, liii, pp. 1864 and 2148.

[3] Bohne, A. Vergleichende bakteriologische Blut-, Stuhl- und Urinuntersuchungen bei Typhus abdominalis. Zeitschr. f. Hyg., etc., 1908, lxi, 213.

doubt that the contamination of streams and reservoirs is a frequent cause of the outbreak of epidemics of typhoid fever, but the actual finding and isolation of the bacilli is a very rare occurrence. This is often due to the fact that the contamination has passed away before the bacteriological examination is undertaken, and also to the great difficulties met with in detecting a few typhoid bacilli when they are associated with large numbers of other bacteria. The greater the amount of contamination entering the water, and the shorter the time which elapses between this and the drinking of the water, the greater is the danger. A recent isolation of the typhoid bacillus is that from the small storage reservoir supplying Scranton. In this city of over 100,000 inhabitants more than 1 per cent. were infected during the epidemic of the winter of 1907. The bacillus was isolated by Fox from about 1 liter of water. Tested alongside of a culture from one of the Scranton cases it seemed identical.

The Importance of Ice in the Production of Typhoid Fever.— We may endeavor to settle this question directly by determining whether epidemics or scattered cases of typhoid fever have been traced to ice, or, failing in this, we may try to estimate the probability of such infection by learning the duration of life of the typhoid bacillus after freezing.

The total number of instances of typhoid fever which have been directly traced to ice infection are remarkably few. One was in France, where a group of officers placed ice made from water polluted by a sewer in their wine and afterward a large percentage developed typhoid fever, while those of the same company not using ice escaped. A second case was a small epidemic which occurred in those who used ice from a pond. It was found that water directly infected with typhoid fæces had flowed over its frozen surface and been congealed there. If typhoid fever is communicated through ice, except under exceptional conditions, it is remarkable that so few cases are traced to it.

The fact that freezing kills a large percentage of typhoid bacilli makes it indeed possible to conceive that ice from moderately infected water contains so few living typhoid bacilli that only the exceptional person here and there becomes infected, and so the source of the infection remains undetected.

If this be true and scattered cases occur, there should be at least some increase on some or every year in March, April, and May in such a city as New York, where four-fifths of all the ice consumed is from the Hudson River, which is known to be contaminated with typhoid bacilli. The people of New York use ice very freely and most of them put it directly in their water or place their vegetables on it. The new ice from the Hudson River is gathered in January or February and stored on top of the left-over ice, and thus shipments to the city are immediately begun. It is an established fact that typhoid bacilli in ice are most abundant during the days immediately after freezing. At the end of two months less than 0.1 per cent. of the original number survive.

If Hudson River ice produced an appreciable amount of typhoid fever, this would then be noticeable in March and in April and perhaps in May.

When we examine the records for the past ten years we find no increase of typhoid fever in Greater New York during those months, with the one exception of 1907, when we had in the borough of Manhattan a sharp outbreak lasting four weeks. This outbreak did not occur at all in Brooklyn. As the people of Brooklyn drank different water, but received ice from the same places of the Hudson River as those of Manhattan, this directed attention to the water or milk rather than the ice. Examination of the Croton watershed at the time showed that a small epidemic of typhoid existed there and that pollution of the water was probable. This suggested still more strongly that the water and not the ice was the cause of the typhoid infection.

It happened that most of the cases occurred in those living in the section of the upper West Side, where only well-to-do people live. An investigation showed that the majority of the infected had used only artificial ice and several had used no ice in their water at all.

Let us now turn our attention to the life of the typhoid bacillus in ice in laboratory experiments. The first important investigation was that of Prudden, who showed that typhoid bacilli might live for three months or longer in ice. This experiment is frequently wrongly interpreted, as when a recent writer states: "It has been amply demonstrated that the germs of typhoid fever are not killed by freezing and that they have been known to live in ice for long periods of time."

It is true that in Prudden's experiment a few typhoid bacilli remained alive for three months, when the experiment was terminated, but those were but a small fraction of 1 per cent. of the original number. Following Prudden's experiment Sedgwick and Winslow in Boston and Park in New York City carried on independently a series of experiments. These led to the same conclusions. A table summarizing a final experiment of ours in which twenty-one different strains, mostly of recent isolation, were subjected to the test is given below:

LIFE OF TWENTY-ONE STRAINS OF TYPHOID BACILLI IN ICE.

	Average number of bacilli in ice.	Percentage typhoid bacilli living.
Before freezing	2,560,410	100
Frozen three days	1,089,470	42
Frozen seven days	361,136	14
Frozen fourteen days	203,300	8
Frozen twenty-one days	10,280	0.4
Frozen twenty-eight days	4,540	0.17
Frozen five weeks	2,950	0.1
Frozen seven weeks	2,302	0.09
Frozen nine weeks	127	0.005
Frozen sixteen weeks	107	0.004
Frozen twenty-two weeks	0	0

In these experiments twenty-one different flasks of Croton water were inoculated each with a different strain of typhoid bacilli. In

one a little of the fæces rich in typhoid was directly added. The infected water in each flask was then pipetted into thirty tubes. These tubes were placed in a cold-storage room in which the temperature varied from 20 to 28° F. At first tubes were removed and tested twice a week, later once a week. The object of using so many different strains was because it has become evident that some cultures live longer than others.

At the end of five weeks the water infected with six cultures was sterile, at the end of sixteen weeks only four strains remained alive.

Interesting investigations of Hudson River ice were carried out in 1907 by North.

There was noticed a considerable difference between the number of bacteria in the top, middle, and bottom layers of ice. This is natural, since while water in freezing from above downward markedly purifies itself, 75 per cent. of the solids and a fair proportion of bacteria being eliminated, yet this cannot happen in the case of the snow blanket which becomes flooded by rain or by cutting holes through the ice. Here all impurities, such as dust and leaves which have fallen on the surface and dirt which may come from the water, remain with the bacteria which they carry, since all are retained in the porous snow. The bacteria in freshly cut bottom ice generally show the least destruction by freezing.

Dr. North, in his investigation, examined the ice from forty spots between Hudson and Albany. He took samples from the top, middle and bottom of each cake and the water of the river.

The river water in the forty specimens averaged 1,800 bacteria per c.c., the top ice 306, the bottom ice 36, and the middle ice 14. Only four specimens of top ice had over 500 bacteria per c.c., none of the specimens of middle or bottom ice.

Thirty-three of the specimens of water had over 500 and 23 over 1000. Colon bacilli were obtained from but one specimen of the middle ice, two from the bottom ice, and twelve from the top ice.

The great destruction by freezing is noticeable in these figures. Even the top ice soiled by the horses and men gathering it contained but 16 per cent. as many bacteria as the water from which it was obtained. The bottom ice, the last to be frozen, had but 2 per cent. of those in the water.

Conclusions in Regard to Ice Pollution.—The danger from the use of ice produced from polluted water is always much less than the use of the water itself. Every week that the ice is stored the danger becomes less, so that at four weeks it has become as much purified from typhoid bacilli as if subjected to sand filtration. At the end of four months the danger becomes almost negligible, and at the end of six months quite so. The slight danger from freshly cut ice, as well as the natural desire not to put even sterilized and diluted frozen sewage in our water, suggests that portions of rivers greatly contaminated, such as the Hudson within three miles of Albany, should be con

demned for harvesting ice for domestic purposes—such ice alone to be used where there is absolutely no contact with food.

Differential Diagnosis.—The typhoid bacillus and the bacilli of the colon group resemble each other in many respects. It is necessary to remember that there are many varieties of bacilli differing in both cultural and agglutinating reactions which are grouped under the general name of the colon bacillus. By comparing what has been said of the *Bacillus coli* and the *Bacillus typhosus* it will be seen that while certain varieties of each simulate each other in many respects, the characteristic varieties of each still possess individual characteristics by which they may be readily differentiated:

1. The motility of the *B. coli* is, as a rule, much less marked than that of the *B. typhosus*. The colon bacillus is also shorter, thicker, and has fewer flagella.

2. In gelatin the colonies of the colon bacillus develop more rapidly and luxuriantly than those of the typhoid bacillus.

3. On potato the growth of the colon bacillus is usually rapid, luxuriant, and visible, though not invariably so; while that of the typhoid bacillus is ordinarily invisible.

4. The characteristic colon bacillus coagulates milk in from thirty-six to forty-eight hours in the incubator, with acid reaction. The typhoid bacillus does not cause coagulation.

5. The colon bacillus is conspicuous for its power of causing fermentation, with the production of gas in media containing glucose. The typhoid bacillus never does this.

6. In nutrient agar or gelatin containing lactose and litmus tincture, and of a slightly alkaline reaction, the color of the colonies of the colon bacillus is pink, and the surrounding medium becomes red; while the colonies of the typhoid bacillus are blue, and there is little or no reddening of the surrounding medium. The same points hold true on the Drigalski-Conradi medium.

7. The colon bacillus possesses the property of producing indol in cultures in bouillon or peptone; the characteristic typhoid bacillus does not produce indol in these solutions.

8. The colon bacillus rarely produces thread outgrowths in properly prepared Hiss plate medium. The typhoid bacillus produces thread outgrowths and smaller colonies in this medium. In the Hiss tube medium the colon bacillus produces either a growth limited to the area inoculated or a diffuse growth streaked with clear lines and spaces. The typhoid bacillus produces a diffuse growth, evenly clouding the entire medium.

9. On the Capaldi medium the colon colonies are more granular and darker than those of the typhoid bacilli.

10. Finally, on adding the typhoid bacilli to the serum of animals immunized to the typhoid bacillus, the typhoid bacilli are found to absorb all the agglutinin acting on the typhoid bacilli, while the colon bacilli absorb little or none of it.

None of these tests alone except perhaps the absorption test can

be depended upon for making a differential diagnosis of the atypical colon bacillus which does not ferment sugars with the formation of gas from the typhoid bacillus or other similar bacilli.

Unfortunately, also, in most of these characteristics certain degrees of variation may often be observed and these may lead to confusion. For instance, the morphology may vary considerably, at times even when growth on the same culture media, and the motility is not always equally pronounced; the flagella may vary; the rapidity of growth may differ, especially between freshly made and old cultures; the grape-leaf appearance of the surface colonies on gelatin, which is usually characteristic, may vary with the composition of the gelatin, at times no typical colonies at all being presented; the threads in the Hiss media may be lacking; in rare instances the typhoid bacillus produces indol; the growth on potato is not to be depended on, often being visible and not characteristic; the virulence of both the bacilli is so little characteristic that it can hardly be used for diagnostic purposes; and finally, the serum test is not to be depended on unless the agglutinins in the serum have been properly tested, for there is abundant agglutinin for some of the colon bacilli in the serum of many untreated animals. This is less true of rabbits than of horses and of young than older animals.

In spite, however, of these difficulties it is very easy sufficiently to identify the typhoid bacillus for all practical purposes. A bacillus which grows typically in the Hiss tube media, and shows agglutination with a high dilution of the serum of an animal immunized to the typhoid bacillus, is in all probability the typhoid bacillus. If this bacillus absorbs the specific typhoid agglutinins it is undoubtedly the typhoid bacillus. The same could probably be said of a bacillus which grew characteristically in glucose bouillon and nutrient gelatin, besides showing the specific serum reaction. A still further test is to inoculate animals with several doses of the dead bacilli whose identification is sought, and note whether there is produced a serum which specifically agglutinates undoubted typhoid bacilli.

CHAPTER XXIII.

THE BACILLUS AND THE BACTERIOLOGY OF TUBERCULOSIS.

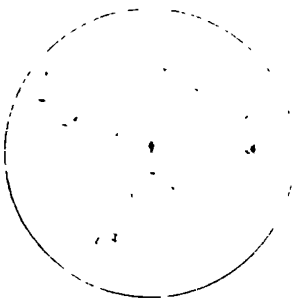
A KNOWLEDGE of phthisis was certainly present among men at the time from which our earliest medical descriptions come. For over two thousand years many of the clearest-thinking physicians have considered it a communicable disease; but it is only within comparatively recent times that the infectiousness of tuberculosis has become an established fact in scientific medicine. Villemin, in 1865, by infecting a series of animals through inoculations with tuberculous tissue, showed that tuberculosis might be induced, and that such tissue carried the exciting agent of the disease. He also noticed the difference in virulence between tuberculous material of human and bovine sources, and says that not one of the rabbits inoculated with human material showed such a rapidly progressive and widespread generalization as those receiving material from the cow. Baumgarten demonstrated early in 1882, bacilli in tissue sections which are now known to have been tubercle bacilli. But these investigations and those of others at the same time, though paving the way to a better knowledge of the disease, proved to be unsatisfactory and incomplete. The announcement of the discovery of the tubercle bacillus was made by Koch in March, 1882. Along with the announcement satisfactory experimental evidence was presented as to its etiological relation to tuberculosis in man and in susceptible animals, and its principal biological characters were given. He submitted his full report in 1884. Innumerable investigators now followed Koch into this field, but their observations served only to confirm his discovery.

Distribution of Bacilli.—They are found in the sputum of persons and animals suffering from pulmonary or laryngeal tuberculosis, either free or in the interior of pus cells; in miliary tubercles and fresh caseous masses in the lungs and elsewhere; in recent tuberculous cavities in the lungs; in tuberculous glands, joints, bones, serous effusions, mucous membranes, and skin affections. They are also found in the fæces of those suffering from tuberculous disease of the intestines or of those swallowing tuberculous sputum.

Morphology.—The tubercle bacilli are slender, non-motile rods of about 0.3μ in diameter by 1.5 to 4μ in length. (Plate I., Figs. 1 and 2.) The morphology is extremely variable, especially on culture media, and varies with the type of medium used. Commonly they occur singly or in pairs, and are then usually slightly curved; frequently they are observed in smaller or larger bunches. Under exceptional conditions branching and club-shaped forms are observed. The tubercle bacillus clearly belongs among the higher forms of bacteria and is closely allied

PLATE I

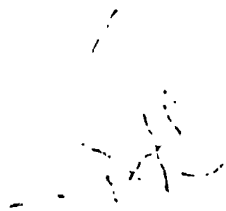
FIG. 1



Tuberculosis bacilli: Human.

× 1000 diameters.

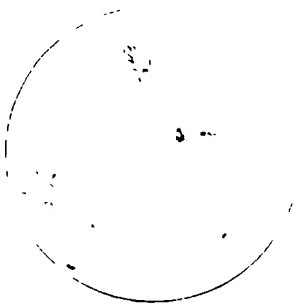
FIG. 2



Tubercle bacilli in red. Tissue in blue.

× 1100 diameters.

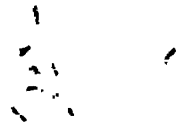
FIG. 3



Leprosy bacilli in nasal secretion of person suffering from nasal lesions. (Hansen.)

× 500 diameters.

FIG. 4



Short smegma bacilli in red, rest of material in blue.

× 1100 diameters.

to nocardia. In stained preparations there are often seen unstained portions. From two to six of these vacuoles may sometimes be noticed in a single rod. In old cultures irregular forms may develop, the rods being occasionally swollen at one end or presenting lateral projections. Here also spherical granules appear which stain with more difficulty than the rest of the bacillus and also retain the stain with greater tenacity. The bacilli, however, containing these bodies are not appreciably more resistant than those not having them; therefore they cannot be considered true spores. (See, however, nocardia.)

The bacilli have a thin capsule, shown in one way by the fact that they appear thicker when stained with fuchsin than with methylene blue. The capsule is believed to contain the greater portion of the wax-like substance peculiar to the bacillus. The characteristics of different stains are given below.

Staining Peculiarities.—These are very important, for by them its recognition in microscopic preparations of sputum, etc., is rendered possible. Owing to the waxy substance in its envelope it does not readily take up the ordinary aniline colors, but when once stained it is very difficult to decolorize, even by the use of strong acids. The more recently formed bacilli are much more easily stained and decolorized than the older forms. The details of methods of staining are given on pages 342, 343.

Biology.—The bacillus of tuberculosis is a *parasitic, aërobic, non-motile* bacillus, and grows only at a temperature of about 37° C., limits 30° to 42° C. It does not form true spores.

Resistance.—The bacilli, on account of the nature of their capsule, it has been assumed, have a somewhat greater resisting power than most other pathogenic bacteria, since frequently a few out of a great number of bacilli resist desiccation at ordinary temperatures for months; most bacilli die, however, soon after drying. This, however, may be the case with any pathogenic organism and it is doubtful if there is a greater resistance shown by the tubercle bacillus than by a considerable number of other non-sporebearing bacilli. Upon cultures the bacilli do not live longer than three months, unless the media be favorable, such as egg or serum; transplants after this time may fail to grow, showing that at least the majority of the bacilli are dead. A few bacilli, sufficient to infect guinea-pigs, may persist much longer. They frequently retain their vitality for several weeks in putrefying material, such as sputum. Cold has little effect upon them. When dry, some of the organisms stand dry heat at 100° C. for twenty minutes but are dead in forty-five minutes; but when in fluids and separated, as in milk, they are quickly killed—viz., at 60° C. in twenty minutes, at 65° C. in fifteen minutes, at 70° C. the great majority in one minute, all in five minutes, at 80° C. the great majority in one-half minute, all in one minute, and at 95° C. in one-half minute. There are reports of experiments which indicate that tubercle bacilli may withstand heat to a greater extent than the above figures indicate. It

is possible when masses of enormous numbers, especially in coagulated clumps, are tested one or two bacilli may resist the exposures noted. One reason why in some experiments they appear to withstand high temperature is, as pointed out by Theobald Smith, that when heated in a test-tube in the usual way the cream which rises on heating is exposed on its surface to a lower temperature than the rest of the milk, and as this contains the greatest percentage of the bacteria some of them are exposed to less heat than those in the rest of the fluid receive. Rosenau points out, however, that where reports seem to indicate that the tubercle bacillus is more resistant than the average pathogenic organism the following is the cause: If a moderate number of killed bacilli are injected, limited lesions will arise and caseation may follow. On killing and autopsying the animals, tubercle bacilli can then be demonstrated in smears from the lesions, and the inoculation is considered positive. If, however, this material is reinjected into a second pig, the latter will show nothing on autopsy. This capacity of dead bacilli to cause macroscopic lesions has long been known, having been shown by Prudden and Hodenpyl. Its importance, however, is not sufficiently considered. Cultures are not suitable to test the viability of the bacillus, inoculations into guinea-pigs are resorted to and another animal should be inoculated from the first one.

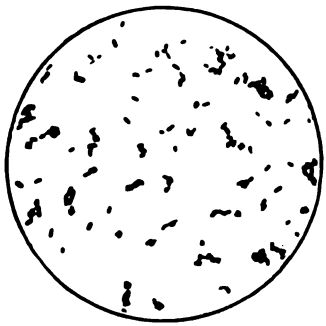
FIG. 107

Tubercle bacilli. Impression preparation from small colony on coagulated blood serum. × 1000.

The resisting power of this bacillus to chemical disinfectants, drying, and light is considerable, but not as great as it is apt to appear, for, as in sputum, the bacillus is usually protected by mucus or cell protoplasm from penetration by the germicidal agent. It is not always destroyed by the gastric juice in the stomach, as is shown by successful infection experiments in susceptible animals by feeding them with tubercle bacilli. They are destroyed in sputum in six hours or less by the addition of an equal quantity of a 5 per cent. solution of carbolic acid. Bichloride of mercury is less suitable for the disinfection of sputum as it combines with the mucus and forms a more or less protecting envelope. Iodoform has no effect upon cultures until .5 per cent. is added. The fumes from four pounds of burning sulphur to each 1000 cubic feet of air space will kill tubercle bacilli in eight hours when fully exposed to the action of the gas, providing they are moist, or abundant moisture is present in the air. Formaldehyde gas is quicker in its action, but not much more efficient. Ten ounces of formalin should be employed for each 1000 cubic feet of air space.

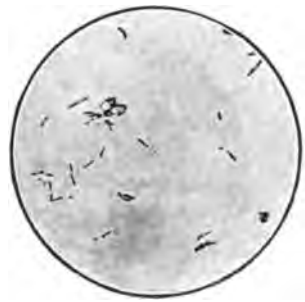
The tubercle bacillus in sputum when exposed to direct sunlight is killed in from a few minutes to several hours, according to the thickness of the layer and the season of the year; it is also usually destroyed by diffuse daylight in from five to ten days when placed near a window in fine powder. Protected in cloth the bacilli survive exposure to light for longer periods. Tuberculous sputum expectorated upon sidewalks, etc., when left undisturbed in the shade may be infectious for weeks, but when exposed to the action of direct sunlight, will in many cases, especially in summer, be disinfected by the time it is in condition to be carried into the air as dust, but not before children

FIG. 108



Tubercle bacilli, bovine. × 1000 diameters.

FIG. 109



Tubercle bacilli, human. × 1000 diameters.

and flies get into it. This action of sunlight and other more important hygienic reasons, suggest that the consumptive patients should occupy light, sunny rooms.

Dried sputum in rooms protected from abundant light has occasionally been found to contain virulent tubercle bacilli for as long as ten months. For a year at least it should be considered dangerous. The Roentgen rays have a deleterious effect on tubercle bacilli in cultures, but practically none upon those in tissues.

Multiplication of Tubercle Bacilli in Nature Takes Place Only in the Living Animal.—The tubercle bacillus is a strict parasite—that is to say, its biological characters are such that it could scarcely find natural conditions outside of the bodies of living animals favorable for its multiplication. Under exceptional conditions, such as in freshly expectorated sputum, tubercle bacilli may increase for a limited time.

Cultivation of the Tubercle Bacillus.—On account of their slow growth and the special conditions which they require, tubercle bacilli cannot be grown in pure culture by the usual plate method on ordinary culture media. Koch first succeeded in cultivating and isolating this bacillus on coagulated beef serum, which he inoculated by carefully rubbing the surface with sections of tuberculous tissue and then leaving the culture, protected from evaporation, for several weeks in the incubator. Cultures are more readily obtained of human than of bovine bacilli.

Growth on Coagulated Dog or Bovine Serum or on Egg.—On these, one of which is generally used to obtain the first culture, the growth is usually visible at the end of ten days at 37° C., and at the end of three or four weeks a distinct and characteristic development has occurred. On serum small, grayish-white points and scales first appear on the surface of the medium. As development progresses there is formed an irregular, membranous-looking layer. On egg the growth is in the form of more or less elevated colonies which may become confluent.

Growth on Nutrient 3–5 per cent. Glycerin Agar.—Owing to the greater facility of preparing and sterilizing *glycerin agar*, it is now usually employed in preference to blood serum for continuing to produce later cultures. When numerous bacilli have been distributed over the surface of the culture medium, a rather uniform, thick, white layer, which subsequently acquires a slight yellowish tint, is developed; when the bacilli sown are few in number, or are associated in scattered groups, separate colonies are developed, which acquire considerable thickness and have more or less irregular outlines. The growth appears similar to that shown upon bouillon as seen in Fig. 110.

Growth on Nutrient Veal or Beef Broth Containing 5 per cent. of Glycerin.—This is of importance, because in this way tuberculin is produced. On these media the tubercle bacillus also grows readily if a very fresh thin film of growth from the glycerin agar is floated on the surface. Glycerin broth is used for the development of tu-

FIG. 110

Growth of tubercle bacilli upon glycerin bouillon. (Kolle and Wassermann.)

berculin and must be neutral to litmus, viz., between 1.5 per cent. to 2 per cent. acid to phenolphthalein. The small piece of pellicle removed from the previous culture continues to enlarge while it floats on the surface of the liquid, and in the course of three to six weeks covers it wholly as a single film, which on agitation is easily broken up and then settles to the bottom of the flask, where it ceases to develop further. The liquid remains clear. A practical point of importance, if a quick growth is desired, is to remove for the new cultures a portion of the pellicle of a growing bouillon culture, which is very thin and actively increasing.

Growth on Potato.—A good growth from cultures and sometimes even from tissue takes place on potato, and this forms the most uniform medium for stock cultures. After the potato is cut, soak in 1–1000 sodium carbonate so-

lution for twenty-four hours, drain, and then soak in 5 per cent. glycerin solution in distilled water for twenty-four hours. Tube and add the glycerin solution for moisture. The potato tubes are paraffined to lessen evaporation and may have at their lower end a bulb to hold sufficient fluid to prevent the potato from drying, though special tubes are not necessary.

Obtaining of Pure Cultures of the Tubercle Bacillus from Sputum, Infected Tissue, and other Materials.—On account of the time required and the difficulties to be overcome, this is never desirable except when careful investigations of importance are to be undertaken. The chief point of present interest is to determine the type of bacillus present in as many cases as possible to learn the frequency of bovine bacilli in man. *Pure cultures* can be obtained directly from tuberculous material when mixed infection is not present, and a proper blood serum or egg culture medium is at hand; but it is difficult to get material free from other bacteria which grow much more rapidly and take possession of the medium before the tubercle bacillus has had time to form visible colonies. Therefore, it is usually necessary first to inoculate guinea-pigs, subcutaneously or intramuscularly, preferably in the thigh, and then obtain cultures from the animals as soon as the tuberculous infection has fully developed. From acute tuberculosis in man in other regions than the lungs, direct cultures on blood serum or egg may be made with some hope of success. Under the best conditions great care and patience are necessary if successful results are to be obtained.

Animals inoculated usually die at the end of three weeks to four months. It is better, however, not to wait until the death of the animals, but at the end of four to six weeks to kill a guinea-pig without violence, using illuminating gas, chloroform, or ether in a closed tin or jar. (Animals which develop tuberculosis acutely are apt to have abundant tubercle bacilli and give successful cultures, while the chronic cases usually have few bacilli and frequently give unsuccessful cultures.) The animal after being killed is tied out in trays, and after washing with a 5 per cent. solution of carbolic acid, immediately autopsied. The skin over the anterior portion of the body having been carefully turned back, the inguinal nodes are removed with fresh instruments. The nodes on the side of injection are especially favorable for cultures. The abdomen is then opened and the spleen and retroperitoneal nodes removed. As the organs are removed they should be placed in petri dishes and thoroughly minced with knife and forceps. Fresh instruments should be used for each operation. The sternal nodes may be used for cultures, but the lungs are almost useless as the majority of cultures will be contaminated. The minced tissue is then placed on the surface of the culture media and evenly and thoroughly smeared over its surface, then the cotton plug is dipped in hot paraffin and the tube corked with a tightly fitting charred cork, to keep the media from drying. The tubes are incubated in the inclined position. On egg, growth is visible in from seven to ten days, and well marked at the end of three weeks. Many tubes should be inoculated as it is only with

the dexterity acquired by practice that contaminations are avoided. As will be noted further on, the growth of the bovine type will be very sparse and on glycerin egg probably negative.

Media for Isolation.—Egg media are the best, failure of growth even when the bacilli are few practically never occurs. This medium was first advised by Dorset, and has been variously modified. Absolutely fresh eggs should be secured. Wash clean of any adherent dirt, then wash with 5 per cent. carbolic, allow to partly dry; then with flamed forceps punch holes in both ends, rupturing the membrane at one end, this end is held over a sterile flask and the contents carefully blown out. If the blowing is done from the cheeks rather than from the lungs, spattering of saliva and blowing into the flask is avoided. To the eggs is then added 10 per cent. of water by volume of the weight of the eggs. The flask is carefully shaken and the contents mixed with a sterile rod. The mixture is then strained through cheese-cloth into a funnel, and tubed. This is done by tying cheese-cloth over a funnel just so it sags about two inches below the rim when pressed. Over this is placed a common pie plate to protect from dust. To the end of the funnel is attached a rubber tube with drawn out piece of glass tubing for a tip and pinchcock put in place. To protect the tip from dust a piece of tubing is used about 3 inches long and wide enough to allow the test-tubes used to slip through. Into one end a perforated rubber cork is inserted and the glass tip pushed through the cork half-way down the larger glass tube. The pinchcock having been loosened the whole is carefully wrapped and sterilized in the autoclave. When ready for use it is carefully unwrapped, set in a ring stand, and the pinchcock tightened. The tin is tilted up and the mixed egg poured upon the cheese-cloth. It filters through by gravity. The tubes to be filled are then flamed and pushed up into the larger tube, and thus filled from the protected tip.

Another modification of the egg medium is that of Lubenau. This consists of 10 eggs plus 200 c.c. of 5 per cent. glycerin bouillon neutral to litmus, treated as above.

After the tubes are filled they are then inspissated at 70° C. for two hours, and incubated for one week for sterility. In coagulating, the air should be thoroughly saturated with moisture, and if the Koch inspissator is used preferably only one layer should be coagulated at a time. After the tubes are inspissated a few drops of sterile water should be added. To prevent evaporation push down the stopper, burn, and plug with a charred cork.

Pathogenesis.—The tubercle bacillus is pathogenic not only for man, but for a large number of animals, such as the cow, monkey, pig, cat, etc. Young guinea-pigs are very susceptible, and are used for the detection of tubercle bacilli in suspected material. When inoculated with the minutest dose of the living bacilli they usually succumb to the disease. Infection is most rapidly produced by intraperitoneal injection. If a large dose is given, death follows in from ten to twenty days. The omentum is found to be clumped together in

sausage-like masses and converted into hard knots, which contain many bacilli. There is no serous fluid in the peritoneal cavity, but generally in both pleural sacs. The spleen is enlarged, and it, as well as the liver and peritoneum, contains large numbers of tubercle bacilli. If smaller doses are given the disease is prolonged. The peritoneum and interior organs—spleen, liver, etc., and often the lungs—are then filled with tubercles. On subcutaneous injection, for instance, into the thigh, there is a thickening of the tissues about the point of inoculation, which may break down in one to three weeks and leave a sluggish ulcer covered with cheesy material. The neighboring lymph glands are swollen, and at the end of two or three weeks may attain the size of hazel-nuts. Soon an irregular fever is set up, and the animal becomes emaciated, usually dying within four to eight weeks. If the injected material contained only a small number of bacilli the wound at the point of inoculation may heal up and death be postponed for a long time. On autopsy the lymphatic glands are found to have undergone cheesy degeneration; the spleen is very much enlarged, and throughout its substance, which is colored dark red, are distributed masses of nodules. The liver is also enormously increased in size, streaked brown and yellow, and the lungs are filled with grayish-white tubercles; but, as a rule, the kidneys contain no nodules. Tubercle bacilli are found in the affected tissues, but the more chronic the process the fewer the bacilli present.

Injection into the thigh is to be preferred for diagnostic purposes, the swelling of the local lymph nodes being then palpable. As soon as this is appreciable the node may be removed with or without killing the pig, the presence or absence of tuberculous lesions noted, and smears made for the detection of tubercle bacilli, thus saving time. It must be remembered that the pig may not show the usual picture of generalized tuberculosis, but only a swelling of the local lymph nodes. Fortunately tubercle bacilli are usually easily demonstrable in smears made from the crushed nodes. If there is any doubt the remaining tissue should be emulsified and reinjected into a second set of pigs. Another point to be considered is that other organisms may, rarely, give a picture impossible to distinguish macroscopically from tuberculosis, as, for instance, streptothrix. To safeguard against error smears should be stained and tubercle bacilli demonstrated.

Rabbits are very susceptible to tuberculosis of the bovine type, less so to that of the human type. This will be given more in detail under the differences between human and bovine tuberculosis.

Monkeys are very susceptible to infection with both types of bacilli. Cats, dogs, rats, and mice are susceptible, the last two usually show no tuberculous lesions, but there is great multiplication of the bacilli in the tissues.

Tubercle Toxins.—These comprise both endotoxins and extracellular poisons. Injections of endotoxins cause necrosis, abscess, and cheesy degeneration of tissues and general cachexia. The extracellular poisons produce fever and an acute inflammatory reaction of the

tissues. These poisons will be considered in detail later in connection with tuberculins.

Action upon the Tissues of the Poisons Produced by the Tubercle Bacillus.—Soon after the introduction into the tissues of tubercle bacilli, either living or dead, the cells surrounding them begin to show that some irritant is acting upon them. The connective-tissue cells become swollen and undergo mitotic division, the resultant cells being distinguished by their large size and pale nuclei. A small focus of proliferated epithelioid cells is thus formed about the bacilli, and according to the intensity of the inflammation these cells are surrounded by a larger or smaller number of the lymphoid cells. When living bacilli are present and multiplying the lesions progress, the central cells degenerate and die, and a cheesy mass results, which later may lead to the formation of cavities. Dead bacilli, on the other hand, unless bunched together, give off sufficient poison to cause the less marked changes only (Prudden and Hodenpyl). Of the gross pathological lesions produced in man by the tubercle bacilli the most characteristic are small nodules, called miliary tubercles. When young, and before they have undergone degeneration, these tubercles are gray and translucent in color, somewhat smaller than a millet seed in size, and hard in consistence. But miliary tubercles are not the sole tuberculous products. The tubercle bacilli may cause diffuse growth of tissue identical in structure with that of miliary tubercles, that is, composed of a basement substance, containing epithelioid, giant, and lymphoid cells. This diffuse tuberculous tissue also tends to undergo cheesy degeneration.

Usual Point of Entrance of Infection.—Infection by the tubercle bacillus takes place usually through the respiratory tract or the digestive tract, including the pharynx and tonsils, more rarely through wounds of the skin.

Tuberculosis may be considered to be caused chiefly by the direct transmission of tubercle bacilli to the mouth through soiled hands, lips, handkerchiefs, milk, etc., or by the inhalation of fine particles of mucous thrown off by coughing or loud speaking, or of tuberculous dust contaminated by sputum or fæces.

Tuberculosis of Skin and Mucous Membranes.—When the skin or mucous membranes are superficially infected through wounds there may develop lupus, ulceration, or a nodular growth. The latter two forms of infection are apt after an interval to cause the involvement of the nearest lymphatic glands, and then finally the deeper structures.

Tuberculosis of Respiratory Tract.—The lungs are the most frequent location of tuberculous inflammation, in spite of the fact that on account of their location they are greatly protected. Most of the bacilli are caught upon the nasal or pharyngeal mucous membranes. Only a small percentage can find their way to the larynx and trachea, and still less to the smaller bronchioles. From the examination of the lungs of miners as well as from experimental tests there is no doubt

but that some of the bacilli find their way into the deeper bronchi. The deeper the bacilli penetrate the more unlikely that they can be cast out. The lungs become frequently infected by indirect ways, for it is now well established that infection taking place through the intestine may find its way by the blood to the lungs and excite there the most extensive lesions with or without leaving any trace of its point of entrance. The nasal cavities are rarely affected with tuberculosis, but more often the retropharyngeal tissue. Tuberculosis of this tissue as well as that of the tonsils is apt to give rise to infection of the lymph nodes of the neck. It is believed that just as bacilli may pass through the intestinal walls to infect the mesenteric nodes, so bacilli may, without leaving any trace, pass through the tonsils to the nodes of the neck.

Primary infection of the larynx is rare. Secondary infection is fairly common. The region of the vocal cords and the interarytenoid space are the special sites attacked.

Infection by Inhalation of Dried and Moist Bacilli.—A common mode of infection is by means of tuberculous sputum, which, being coughed up by consumptives, is either disseminated as a fine spray and so inhaled, or, carelessly expectorated, dries and, broken up by tramping over it, sweeping, etc., distributes numerous virulent bacilli in the dust. As long as the sputum remains moist there is no danger of dust infection, but only of direct contact; it is when it becomes dry, as on handkerchiefs, bedclothes, and the floor, etc., that the dust is a source of danger.

A great number of the expectorated and dried tubercle bacilli undoubtedly die, especially when exposed to the action of direct sunlight; but when it is considered that as many as five billion virulent tubercle bacilli may be expectorated by a single tuberculous individual in twenty-four hours, it is evident that even a much smaller proportion than are known to stay alive will suffice in the immediate vicinity of consumptives to produce infection unless precautions are taken to prevent it. The danger of infection is greatest, of course, in the close neighborhood of tuberculous patients who expectorate profusely and indiscriminately, that is, without taking the necessary means for preventing infection. We found that of 100 tuberculous men admitted to one of the consumption hospitals, only 20 claimed to have taken any care to prevent the contamination of their surroundings by their sputum. There is much less danger of infection at a distance, as in the streets for instance, where the tubercle bacilli have become so diluted that they are less to be feared. In rooms the sputum is not only protected from the direct sunlight, but it is constantly broken up and blown about by the walking, closing of doors, etc. In crowded streets on windy days infected dust must sometimes be in the air unless the expectoration of consumptives is controlled.

Exhaustive experiments made by many observers have shown that particles of dust collected from the immediate neighborhood of consumptives, when inoculated into guinea-pigs, produce tuberculosis

in a considerable percentage of them; whereas, the dust from rooms inhabited by healthy persons or dust of the streets does so only in an extremely small percentage. Flügge is probably right in thinking that the dust which is fine enough to remain for a long time in suspension in the air is usually free from living bacilli. It is in the coarser though still minute particles, those in which the bacilli are protected by an envelope of mucus, that the germs resist drying for considerable periods. These are carried only short distances by air currents. Such results as those obtained by Straus, who, on examining the nasal secretions of twenty-nine healthy persons living in a hospital with consumptive patients, found tubercle bacilli in nine of them, must be accepted with some reserve, since we know that in the air there are bacilli which look and stain like tubercle bacilli and yet are totally different. It may be said that the danger of infection from slight contact with the tuberculous is not so great as it is considered by many, but that on this account it is all the more to be guarded against in the immediate neighborhood of consumptives. Those who are most liable to infection from this source are the families, especially young children, the nurses, the fellow-workmen, and fellow-prisoners of persons suffering from the disease. In this connection, also, attention may be drawn to the fact that rooms which have been recently occupied by consumptives are not infrequently the means of producing infection (as has been clinically and experimentally demonstrated) from the deposition of tuberculous dust on furniture, walls, floors, etc. The danger is not apt to last beyond three months. Flügge has recently drawn attention to the fact that in coughing, sneezing, etc., very fine particles of throat secretion containing bacilli are thrown out and carried by air currents many feet from the patient and remain suspended in the air for a considerable time. To encourage us we now have a mass of facts which go to show that when the sputum is carefully looked after there is very little danger of infecting others except by close personal contact.

Tuberculosis of Digestive Tract.—Tuberculosis of the gums, cheeks, and tongue is rare. The tonsils and pharynx are somewhat more often involved. The stomach and œsophagus are almost never attacked. The small intestines are rather frequently the seat of infection from bacilli swallowed with the food or dust-infected mucus. In a striking case four previously healthy children died within a short period of one another. Their nurse was found to have tuberculosis of the antrum of Highmore, with a fistulous opening into the mouth. She had the habit of putting the spoon with which she fed the children into her mouth so as to taste the food before it was given to them. As already noted, the bacilli frequently pass through the mucous membrane to the lymph glands without leaving any lesions.

Infection by Ingestion of Milk and Milk Products.—Milk serves as a conveyer of infection, whether it be the milk of nursing mothers or the milk of tuberculous cows. In this case evidence of infection is usually shown in the mesenteric and cervical lymph nodes or general-

ized tuberculosis may be caused, while the intestinal walls are frequently not affected. Bacilli accompanied by fat much more readily pass through the intestinal mucous membrane or that of the tonsils and pharynx. The transmission of tubercle bacilli in the milk of tuberculous cows has been abundantly proved.

Formerly it was thought that in order to produce infection by milk there must be a local tuberculous affection of the udder; but it is now known that tubercle bacilli may be found in the milk in small numbers, when adjacent tissue is infected and when careful search fails to detect any udder disease. Schroeder has shown that the fæces are a very dangerous factor in the dissemination of tubercle bacilli. He compares fæces in cattle to sputum in man, since the tubercle bacilli are swallowed by cattle and are to a great extent passed through the intestinal tract without destruction. He found that when milk from phthisical cows having healthy udders was obtained so as not to become infected by feces it was free from bacilli, but when obtained without special precautions it was frequently infected. The milk of every cow which has any well-developed internal tuberculous infection must therefore be considered as possibly containing tubercle bacilli. Rabinowitsch, Kempner, and Mohler also proved beyond question that not only the milk of tuberculous cattle, which showed no appreciable udder disease, but also those in which tuberculosis was only detected through tuberculin, frequently contained tubercle bacilli. Different observers have found tubercle bacilli in 10 to 30 per cent. of the samples of unheated city milk. Butter may contain tubercle bacilli in higher percentages of samples examined. When we consider the prevalence of tuberculosis among cattle we can readily realize that, even if the bovine bacillus infects human beings with difficulty, there is danger to children when they are exposed to this source of infection. The milk from cattle suffering from udder tuberculosis usually contains a few hundred bacilli per c.c., but may contain many millions. It is also important to mention the fact that mixed milk from a herd, though tending to dilute the milk of cows excreting tubercle bacilli, may be badly infected from one cow, especially if this cow has udder disease.

Taking the abattoir statistics of various countries, we find that about 10 per cent. of the cattle slaughtered were tuberculous. A less probable source of infection by way of the intestines is the flesh of tuberculous cattle. Here the danger is considerably less, from the fact that meat is usually cooked, and also because the muscular tissues are seldom attacked. In view of the finding of the bovine type of bacilli in a considerable percentage of the few cases of tuberculous children tested, the legislative control and inspection of cattle and milk would seem to be an absolute necessity. As a practical and simple method of preventing infection from suspected milk, sufficient heating of the milk used as food must commend itself to all. Human tubercle bacilli may be found in milk as instanced by one sample of city milk examined in the Research Laboratory by Hess.

Method of Examining Milk for Tubercle Bacilli.—Thirty c.c. of milk are centrifuged at high speed and 10 c.c. of the lower milk and sediment collected. Four cubic centimetres of the cream is thinned with a little sterile water and injected into two guinea-pigs. The sediment is injected in amounts of 3 to 5 c.c. into other pigs. Larger amounts than this are apt to kill too many pigs from the associated bacteria. Subcutaneous injection is to be preferred. There are certain precautions that must be taking in drawing conclusions as the different types of acid-fast "butter bacilli" may cause lesions, and their presence will be noted in smears made from these lesions. To avoid this source of error, two methods are resorted to. If cultures are made from the suspected lesions on glycerin agar, these bacilli develop in a few days, whereas tubercle bacilli would not. When one is ready to kill the pigs, 2 c.c. of old tuberculin should be injected into each pig late in the day. The following morning the tuberculous pigs will be dead or dying. Autopsies should be done on all to confirm the test. The milk should be as fresh as possible to prevent the growth of bacteria.

Bovine Infection in Man.—Numerous investigations have been made on this point. To Ravenel probably belongs the credit of isolating the first bovine bacillus from a child. The following tables summarizing the results of three large series of cases give a fair idea of incidence of such infection. As will be seen, children are especially the ones infected, and usually the point of entry is clearly alimentary as shown by the lesions. Cervical adenitis and abdominal tuberculosis are the most frequent types of infection. Generalized tuberculosis due to bovine infection is less frequent. Bone and joint tuberculosis is almost exclusively of the human type. The meninges are less commonly affected by the bovine type than by the human type. Infection of adults is very uncommon; and, though cases of pulmonary tuberculosis due to the bovine type of bacillus have been reported, the evidence advanced is open to question. Such cases are certainly exceedingly rare.

A careful study of all the factors leads us to estimate that about 10 per cent. of all tuberculosis in children under five is due to bovine infection.

The following tables give a summary of the results obtained in the larger investigations so far carried out:

TABLE I.

TABULATION OF CASES[1] REPORTED BY KOSSEL WEBER, HEUSS, AND TAUTE, AND OEHLECKER (KAISERLICHES GESUNDHEITSAMT) AND BY THE ENGLISH ROYAL COMMISSION.[2]

Diagnosis of cases examined	Adults 16 yrs. and over		Children 5 to 16 yrs.		Children under 5 yrs.		Notes
	Human	Bovine	Human	Bovine	Human	Bovine	
Pulmonary tuberculosis.	22	.....	1	.....	5	.....	Cases diagnosed clinically or by autopsy. Some showed abdominal lesions (ingestion?) but no true generalization. *One case* (age?) = human type.
Tuberculous adenitis (axillary).	.....	.....	.....	.....	1	.....	
Tuberculous adenitis (cervical).	2	.....	8	5	7	4	*One case*, no age, = human type.
Abdominal tuberculosis.	6	.....	2	4	3	7	Lesions exclusively of abdominal organs as far as known.
Generalized tuberculosis (alimentary origin).	3	See notes	2	2 See notes	6	4	Including cases where generalization has begun or is complete. *One case* (30 yrs.) gave both types in mesenteric nodes, human type in bronchial nodes. *One case*, 5½ yrs., gave human type from spleen, bovine type from mesenteric nodes.
Generalized tuberculosis including meninges (alimentary origin).	.....	.....	1	.....	1	6 See notes	Two of the bovine cases had cultures from the meninges. *One case*, 4 yrs., gave human type from meninges and bronchial nodes, bovine from mesenteric nodes.
Generalized tuberculosis.	15	.....	.....	1	3	.....	Pulmonary lesions predominant in most of cases.
Generalized tuberculosis incl. meninges.	.....	.....	3	.....	10	.....	Eight cases had cultures from the meninges.
Tuberculosis of bones and joints.	16	.....	15	1	14	.....	One case, age not stated, gave human type.
Genitourinary tuberculosis.	4	.....	.....	.....	.....	.....	
Tuberculosis of skin.	1	.....	1	.....	.....	.....	
Miscellaneous	1	.....	.....	.....	.....	.....	Calcified mesenteric and bronchial nodes. Carcinoma.
Totals	70	..... See notes	33	13 See notes	50	21 See notes	(6 non-tabulated) Total cases 193.

[1] The cases were selected, that is, to include as many cases of alimentary origin as possible.

[2] Exclusive of sputum feeding, two cases without culture and cases showing irregularity or change in virulence, for which refer to original.

TABLE II.

THE RELATIVE PROPORTION OF HUMAN AND BOVINE TUBERCLE BACILLI INFECTIONS IN A LARGE SERIES OF UNSELECTED CASES[1] EXAMINED AT THE RESEARCH LABORATORY.

Diagnosis of cases examined	Adults 16 yrs. and over		Children 5 to 16 yrs.		Children under 5 yrs.		Notes
	Human	Bovine	Human	Bovine	Human	Bovine	
Pulmonary tuberculosis.	278	.....	8	.....	5	.....	Clinical diagnosis only known, and therefore no positive details as to extent of tuberculosis elsewhere than in lungs.
Tuberculous adenitis, inguinal and axillary.	1	.....	4	.....	.....	.....	(See next.)
Tuberculous adenitis, cervical.	9	.....	19	8	6	12	In two cases cultures were from axillary nodes, but primary focus was cervical. One case died shortly afterward with pulmonary tuberculosis.
Abdominal tuberculosis.	1	.....	1	1	.....	3	
Generalized tuberculosis, alimentary origin.	.....	.....	.....	.....	1	1	Only two cases are given under this heading. Many of the cases in the following subdivisions showed marked intestinal lesions and some possibly were of alimentary origin.
Generalized tuberculosis.	2	.....	1	.....	12	4	One bovine case had tuberculous osteomyelitis of metatarsal bone.
Generalized tuberculosis including meninges.	.....	.....	.....	.....	18	1 See notes	*One case* not included in table gave both types of bacillus.
Tubercular meningitis.	.....	.....	1	.....	14	1	No autopsy. Extent of lesions elsewhere, unknown.
Tuberculosis of bones and joints.	1	.....	10	.....	6	.....	
Genitourinary tuberculosis.	3	1	1	.....	.....	.....	
Tuberculous abscesses.	1	.....	.....	.....	.....	.....	Possibly primary in bone.
Totals	296	1	45	9	62	22 See notes	(1 non-tabulated) Total cases 436.

[1] Unselected cases from the hospitals of New York City.

Hypothesis of Transmissibility of Tubercle Bacilli to the Fœtus.—There is some evidence of the transmission of tubercle bacilli from the mother of the fœtus in animals. With regard to tuberculosis in the human fœtus the evidence is not so clear, though some twenty cases have been recorded of tuberculosis in newly born infants, and about a dozen cases of placental tuberculosis. As to the infection of the fœtus from the paternal side, where the father has tuberculosis of the scrotum or seminal vessels, we have no reason to suppose that such can occur. There are, however, grounds for belief that infection in this way may take place from husband to wife. Thus, Gärtner found, as a result of his experiments in animals, that a large majority of the guinea-pigs and rabbits which were brought together with males whose semen contained tubercle bacilli died of primary genital tuberculosis; but from the rarity of this affection in women and cows it may be assumed that tubercle bacilli occur very much less frequently in semen of men and cattle than in that of the smaller animals.

Attenuation.—Tubercle bacilli when subjected to deleterious influences or to growth on culture media very slowly decrease in virulence. Cultures grown at temperatures of 42° C. become attenuated more quickly.

Mixed Infection.—In regions where tuberculous processes are on the surface, such as lung and skin infections, and also when the infection itself is multiple, as in disease of the glands of the neck from tonsillar absorption, the tubercle bacilli are frequently associated with one or more other varieties of organisms. Those of most importance are the streptococcus, pneumococcus, and influenza bacillus. Besides these many other varieties are met with occasionally in individual cases. What the influence of this secondary or mixed infection is, under all circumstances, is not exactly known; but generally the effect is an unfavorable one. For the technique employed in examining sputa for mixed infection see page 344.

Individual Susceptibility.—It is believed by many that in demonstrating the possibility of infection in pulmonary tuberculosis its occurrence is sufficiently explained; but they leave out another and most important factor in the production of an infectious disease—individual susceptibility. That this susceptibility, or "predisposition," as it is improperly called, may be either inherited or acquired is now an accepted fact in medicine. It is even thought that the physical signs and characters—the *phthisical habit*—which indicate this susceptibility can be externally recognized. At first the inherited susceptibility was considered more important than the acquired, but now much that was attributed to the former is known to be explained by the fact of living in an infected area. The acquired susceptibility may arise from faulty physical development or from depression, sickness, overwork, excessive use of alcohol, etc. Unquestionably, vast differences exist in different individuals in the intensity of the tuberculous process in the lung. That this does not depend chiefly upon a difference in virulence of the infection is evident from the fact

that individuals contracting tuberculosis from the same source are attacked with different severity, and that there is, as a rule, no great difference in degrees of virulence for animals in the tubercle bacilli obtained from different sources. As is seen from the results of post-mortem examinations in which, according to the completeness of the examinations, the remains of old tuberculous processes have been found in the lungs of about one-third to one-half of all the bodies examined, many cases of pulmonary infection must occur without showing any visible evidence of disease, and heal of their own accord. The possibility of favorably influencing in many an existing tuberculosis by treatment also proves that, under natural conditions, there is a varying susceptibility to the disease. Clinical experience teaches, likewise, that good hygienic conditions, pure air, good food, freedom from care, etc., increase immunity to phthisis. Animal experiments have shown that not only are there differences of susceptibility in various animal species, but also an individual susceptibility in the same species. The doctrine of individual susceptibility, therefore, is seen to be founded on fact, although the reasons for it are only partially understood.

Various other tuberculous affections which are natural in man have been produced experimentally in animals, as, for instance, tuberculosis of the joints, tuberculous abscess, etc.

Immunization.—As in other infectious diseases, many attempts have been made to produce an artificial immunity against tuberculosis, but so far the results have been only fairly satisfactory. The great majority of mankind has in a varying degree some natural immunity against tuberculosis. Even the infant receives through the placental membranes a considerable amount of any immune substances present in its mother. In many individuals this immunity is only relative, and is maintained only as long as the health is kept at a high standard or the exposure to infection not too intense or prolonged. An unfavorable environment, the occurrence of some other infectious disease, overwork, dissipation, or, in fact, anything which tends to depreciate the nutrition of the body, is apt to render the individual, previously immune, susceptible to the tubercle bacillus.

Acquired immunity against many bacterial diseases occurs within a very few days or weeks after the development of infection. This immunity may be complete or slight and vary greatly in its duration. There is little at first glance in the clinical history of tuberculosis which shows that acquired immunity occurs in this disease, for relapse is the rule, and one attack does not seem to afford any protection against a later one. For this reason the production of an artificial immunity against tuberculosis has always been looked upon by many as a result possibly never to be achieved. The careful study of tuberculosis seems, however, to indicate an attempt on the part of nature at the production of acquired immunity in this disease. It is known that from 30 to 60 per cent. of cadavers show the healed lesions of tuberculosis. The small proportion of those which progressed to

serious lesions or became reinfected indicates a degree of acquired immunity.

Artificial immunity is an attempt to imitate nature's methods, and is obtained by the inoculation of a modified living culture or of toxins and dead bacilli. The injection by Koch of the heat-resistant-toxins, as in his original tuberculin treatment, produced in animals a certain degree of acquired resistance to larger doses of toxins, but did not protect to any appreciable degree from subsequent living tubercle bacilli, or produce in animals an antitoxic serum. In 1892 Trudeau succeeded in producing in rabbits an appreciable immunity by inoculations of living avian cultures. The rabbits so treated supported, as a rule, inoculation of virulent tubercle bacilli in the anterior chamber of the eye, while in controls the eyes were invariably lost. Later, attenuated human cultures were used with the same results. De Schweinitz, McFadyan, Behring, Calmette and Pearson and Gillilaud have since reported successful results. The latter two treated a number of cows by giving each of them intravenous injections of 1 to 6 c.c. of an emulsion of human tubercle bacilli. This was of an opacity equal to a twenty-four-hour broth culture of typhoid bacilli. They report from their investigations[1] that the treatment had the effect not only of keeping in check the progress of the tuberculous process, but of causing in some cases a distinct retrogression. The bacilli remained alive in the encapsulated lesions. Calmette fed calves with tubercle bacilli and found that a very small amount of infectious material became arrested in the intestinal and mesenteric glands and resulted in immunity, while a larger amount, though arrested for a time, later passed through and caused a general and fatal infection. Behring has had prepared an emulsion of attenuated human bacilli to use in cattle. This should be used in cattle within a month of its preparation since the bacilli gradually die and lessen the effect of the vaccination.

The work already done is believed by Trudeau to establish the principle that the most successful protective inoculation is the living germ of such diminished virulence for the animal experimented upon as to produce a reaction ending in healing of the process at first set up by it. This is termed by Behring isopathic immunity. After the living culture the best results have been obtained with the unheated filtrate of bouillon cutlures or the ground-up protoplasm of the chemically unaltered bacilli.

The avian and bovine bacilli immunize against infection from human bacilli probably nearly as well as the attenuated human variety. This is strong evidence in favor of the genetic unity of all tubercle bacilli.

The importance of time in the production of artificial immunization has also been thoroughly demonstrated. It seems that whatever degree of immunity it is possible to produce is produced only very slowly. Von Behring found that his vaccinated cows which received the virulent inoculation before three months had passed showed little

[1] University of Pennsylvania Medical Bulletin, April, 1905.

immunity and generally died of the infection, while after three months they resisted a fatal dose of the virus. While the principle of artificial immunity seems to be fairly well established by animal experimentation, it must be admitted that the laboratory evidence which bears on the production of immunity in animals, or the cure of experimental tuberculosis by tuberculin, is far from satisfactory.

Trudeau obtained the best results in treating animals with tuberculin in the eye tuberculosis of the rabbit, which is naturally a chronic and almost always a purely localized process. Tuberculosis in the guinea-pig, on the other hand, is an acute progressive infection, and experimental and clinical evidence are in perfect accord in demonstrating that against the acute types of tuberculous infection tuberculin is powerless, whether it be employed in man or animals.

The duration of immunity, such as has been successfully produced so far in animals, has not yet been definitely ascertained, but the evidence so far at hand points to the fact that as the most solid immunity is produced by living though attenuated cultures, the immunity which lasts the longest is also brought about in this way, the antitoxic immunity produced by bacterial products being of shorter duration. The period of immunity after inoculation probably lasts more than one year, but usually less than two years.

Chemical Constituents of Tubercle Bacilli.—The bacilli contain on an average 86 per cent. water. The dry substance consists of material soluble in alcohol and ether, of proteid substance extracted by warm alkaline solutions, and of carboydrates and ash. The alcohol-ether extract equals about one-quarter of the dry substance and consists of 15 per cent. of a fatty acid, which is mostly combined with an alcohol to make a wax. No glycerin is present and, therefore, no true fat. It is on the presence of this wax that the staining characteristics depend. Other substances produce abscess, necrosis, and cheesy degeneration. Lecithin and a convulsive poison are also present in the extract.

The substances left after the ether-alcohol extraction are mostly proteid substances. A nucleic acid which contains phosphorus is present. This is considered by many to be the specific endotoxin of the tubercle bacillus.

Diagnostic or Immunizing Substances Prepared from Tubercle Bacilli or their Cultures.—Tuberculin Original "T. O." (Koch's).—This tuberculin contains not only the products of the growth of the tubercle bacilli in the nutrient bouillon which withstand heat as well as substances extracted from the bodies of the bacilli themselves, but also the materials contained in the bouillon, which have remained unaffected by the activities of the bacilli.

This *tuberculin* is prepared as follows: The tubercle bacillus is cultivated in an infusion of calf's flesh, or of beef flesh, or extract to which 1 per cent. of peptone and 3 to 5 per cent. of glycerin have been added, the culture liquid being slightly alkaline. The inoculation is made upon the surface from a piece of very thin pellicle from

a young bouillon culture, or, if the bouillon culture is unobtainable, with small masses from a culture on glycerin agar. These masses, floating on the surface, give rise in from three to six weeks, according to the rapidity with which the culture grows, to an abundant development and to the formation of a tolerably thick and dry, white crumpled layer, which finally covers the entire surface. At the end of four to eight weeks development ceases, and the layer after a time sinks to the bottom. Fully developed cultures, after having been tested for purity by a microscopic examination, are poured into a suitable vessel and steamed in an Arnold sterilizer for three hours. The bacilli are then filtered off and the liquid evaporated to one-tenth of its original bulk over a water-bath at a temperature of 70° to 100° C. The liquid is then filtered through chemically pure filter-paper and finally through a stone filter. The crude tuberculin thus obtained contains 30 to 50 per cent. of glycerin, albumoses, traces of peptone, extractives, and inorganic salts. The true nature of the toxic substances is not known. It keeps well, retaining its activity indefinitely. When used it is diluted with one-fourth per cent. carbolic acid solution. This diluted tuberculin is not quite stable and should be used within a week's time. It is considered that 1 mg. of tuberculin equals 1 c.c. of a 1:1000 dilution.

Tuberculin Precipitation "T. P."—A quantity of old (concentrated) Koch's tuberculin is poured into two volumes of 95 per cent. alcohol, allowed to settle, and filtered per paper. The sediment is washed with 70 per cent. alcohol until the filtrate runs clear, then pressed between layers of filter-paper to remove excess of moisture, scraped into a dish, dried *in vacuo* over H_2SO_4, and broken up in a mortar. For the Calmette eye test solutions of the powder are made in sterile normal salt solution of ½ and 1 per cent. by weight, boiled in a water-bath, filtered, diluted as required, distributed into small tubes containing about two drops, which are then sealed and boiled for ten minutes.

Bacillus Emulsion "B. E."—This is Koch's latest product. It is an emulsion of the entire substance of the unaltered tubercle bacilli in 20 per cent. of glycerin. The broth culture is poured into a filter and the broth filtered off. The bacilli are washed, pressed between absorbent paper and dried in exsiccator. They are then ground in a mortar until no formed bacilli are found on staining. The powder is taken up in 0.8 per cent. salt solution and added to 20 per cent. glycerin water so that 1 mg. of powder is contained in 0.2 c.c. of the final preparation. Dilutions are made in 0.5 per cent. carbolic acid in 0.8 salt solution. As can be readily seen, in a preparation thus made, contamination is difficult to avoid, freedom from intact bacilli is uncertain. This preparation is, therefore, before marketing, usually subjected to heating at 60° C.

Bouillon Filtrate Tuberculin.—This is the unheated filtrate from bouillon cultures of human tubercle bacilli. Its use was suggested by Denys. The last two preparations are for treatment only. After

six years of trial in the treatment of cases the results obtained from the use of the new tuberculin preparations, which are unheated or heated not over 60° C., are considered superior to those obtained from the older product.

Many other tuberculins have been proposed during the past fifteen years, among which are Hunter's Modification B., von Ruck's Watery Extract, Landemann's Tuberculol, Denys' Bouillon Filtrate, Baraneck's Tuberculins, Spengler's Bovine B. F., and Behring's T. C. and Tulasa, which he claims immunizes cows as well as the living bacilli, but the value of which has not yet been put to a practical test in the treatment of human tuberculosis. These tuberculins are all vaccines, they are all made from either the body substance of the germ or the liquid medium in which it has grown, or both, and their aim is to stimulate the defensive resources of the system, and to induce antitoxic and antibacterial immunity. They all produce, when given in sufficient doses, local reactions in tuberculous foci, and the well-known but little understood phenomena of general tuberculin reaction. These preparations are described in detail by Baldwin in Osler's "Practice of Medicine" (Vol. iii, page 160).

The Use of Tuberculins in Treatment and Immunization.—Koch's old tuberculin, which was at first principally used, has of late been generally discarded for preparations which have not been subjected to heat at least not above 60°. The two most used are B. F., a filtrate of human cultures of recorded virulence to which a quarter of 1 per cent. carbolic acid has been added, or B. E., which is an emulsion in glycerin and water of the pulverized bodies of the virulent tubercle bacilli. With the B. E. habituation takes place with much more difficulty than with B. F., and occasionally unexpected and sometimes violent reactions occur, even if the utmost caution in increasing the dose is exercised. It is possible that having obtained a certain degree of antitoxic immunity with a course of B. F., a secondary course in which B. E. is employed might prove more efficacious, and it is evident we have much yet to learn about the production of the tuberculous vaccines and their application in the treatment of disease. Time and experience alone can show us which tuberculin produces the best results.

According to Koch, the substances produced in the body by the old tuberculin neutralize the tuberculous toxins, but are not bactericidal. After a series of experiments he considered the difficulty to be due to the nature of the envelope of the tubercle bacillus, which made it difficult to obtain the substance of the bacilli in soluble form without so altering it by heat or chemicals that it was useless to produce immunizing substances. He conceived that immunity was not produced in man for somewhat similar reasons—possibly the bacilli never giving out sufficient toxin to cause curative substances to be produced. He therefore decided to grind up the washed and dried bacilli and soak them in water, and thus obtain, if possible, without the addition of heat, a soluble extract of the body substance of the

bacilli, which he hoped would be immunizing. He also tried to eliminate as much as possible of the toxic products which produce fever. Buchner by a different method, through crushing under a great pressure tubercle bacilli mixed with sand, and thus squeezing out their protoplasm, obtained a very similar substance called plasmine. Vaughan has tried the immunizing effect of the non-poisonous split products obtained from treated tubercle bacilli.

Trudeau and others have formulated a schedule by which the initial dose, the intervals between injections, the rate of progression, and the ultimate dose to be attained are distinctly laid down, this schedule to be literally followed so long as the patient shows no evidence of intolerance, but modified at once, as soon as he does, to suit the requirements of each case. Many patients can be carried from beginning to end of the treatment—a period which, when no reactions occur, usually takes about eight months—without any symptoms which call for any departure from the schedule itself, laid down. If this were always so the treatment would be simplicity itself, but unfortunately in the majority of cases, at some period in the treatment, sometimes at the very beginning, sometimes at the middle, and sometimes even at the very last dose, symptoms of intolerance appear, and it is then that the physician requires certain definite rules to guide him in his conduct of the case.

Experience has shown that it is essential to begin treatment with very small doses; that is, for afebrile cases, $\frac{1}{10000}$ milligram of filtrate B. F., or Koch's B. E. (liquid measure, not solid substance), or $\frac{1}{1000}$ milligram of old tuberculin. Denys makes use of eight solutions in giving B. F. No. 1 contains $\frac{1}{10000}$ milligram to each cubic centimeter. This is for febrile cases only. No. 2 contains $\frac{1}{1000}$ milligram to each cubic centimeter; No. 3, $\frac{1}{100}$; No. 4, $\frac{1}{10}$; No. 5, 1 milligram; No. 6, 10 milligrams; No. 7, 100 to each c.c., and No. 8 is pure filtrate. Now the increase in using these solutions is always by 1 decigram of each solution, which is convenient to measure and easy to remember. As 10 decigrams, or 1 c.c. of each solution is reached, the next solution, which is ten times stronger and in which 1 decigram represents the same dose as 1 c.c. of the preceding solution, is taken up and the increase is again by 0.1 of the new solution until 1 c.c. is given when the next solution is taken up in the same way until the end of the treatment. Thus for ten doses the increase for each dose is by $\frac{1}{100000}$ milligram for ten doses; then by $\frac{1}{10000}$ for ten doses; then by $\frac{1}{1000}$ for ten doses; then by $\frac{1}{100}$ for ten doses; then by $\frac{1}{10}$ for ten doses; then by 1 milligram for 10 doses; then by 10 milligrams for 10 doses; then by 100 milligrams, until 1 c.c. of the pure filtrate or old tuberculin is reached. The increase is by 0.1 of each solution, and as each solution is 10 times stronger than the preceeding, the progression in doses is ten times greater at the end of every ten doses. Approximately the same plan may be followed by giving Bacillen-emulsion, provided it is remembered the doses referred to in the above schedule are liquid measure and not solid substance.

Dr. Brown has found that at the Adirondack Cottage Sanitarium reactions occur more frequently at the second or third injection of a new solution. This is not to be wondered at, as the increase in dose is ten times larger when a new solution is taken up. To obviate this, instead of increasing 0.1, 0.2, 0.3, and so on to 10 decigrams, the increase may be 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.6, 0.8, 0.10.

The intervals between the injections are three or four days; generally two injections a week; but as the higher doses, such as 10 milligrams, are reached, the intervals may be five days, and after 100 milligrams six days, while the last three or four doses should be given a week or ten days apart. Lowenstein finds that for Bacillen-emulsion longer intervals are necessary between the doses, especially when the large amounts are reached.

If no intolerance is manifested the treatment will require six months; but in the majority of cases when any reactions occur it should be extended over ten months or a year, or even much longer, if necessary, to reach full doses. It is a mistake to try to shorten the time by increasing the doses too rapidly or decreasing the intervals. Whatever degree of immunity, antitoxic or otherwise, is produced by the treatment is produced only very gradually, and besides the risk to the patient which is always involved by haste, the intolerance it may produce takes often so long to overcome that the duration of the treatment is lengthened rather than shortened in the end.

Tolerance to tuberculin is an excellent prognostic sign and it bears a certain relation to the condition of the patient's general health; and the more this improves the less apt is he to develop symptoms of prolonged intolerance, but the improvement in the general health is necessarily a slow process.

How does intolerance show itself, and how are we to proceed when it does? The symptoms of intolerance may be divided into three groups: those of a general fever reaction, those which indicate local reaction, both at the site of disease and also the site of injection, and those which point to general constitutional disturbance, as manifested by malaise, headache, sleeplessness, wandering pains, anorexia, nausea, and loss of weight and strength.

The fever reactions are of two kinds: the short and the prolonged reaction. The short reaction is identical with that produced by the tuberculin test, and shows the classical fall and rise of temperature all ending in forty-eight hours; the prolonged reaction begins generally more gradually; the symptoms are mild; the fever rises less high but maintains itself, with a morning remission, above the patient's normal temperature range for several days, generally not more than a week.

Local reaction at the site of the lesion is a valuable guide to dosage; increased cough and expectoration, pleuritic pains, aggravation of the physical signs, hoarseness and aphonia if the larynx is involved and pain if a joint, all point to local reaction, and are all indications for caution in increasing the dosage.

Local reaction at the site of injection shows itself by more or less

extensive redness, œdema, and pain; when slight, it may be disregarded, as it is somewhat influenced by the manner of injection or other causes; but if marked, it indicates commencing intolerance and should be considered in connection with the patient's other symptoms before increasing the dose.

Most important, and most often disregarded because no rise in temperature may be present, is the group of symptoms which point to constitutional impairment resulting from overdosage. They are all the symptoms that chronic toxemia might be expected to produce, and all point to the supposition that the patient cannot respond by the formation of antitoxins and antibodies to the increasing doses of toxin which he is receiving. Even if no fever above the usual range be present, the patient who has been improving, and whose general condition has been satisfactory, may show marked arrest in his improvement; if the injections are persisted in and the dose steadily increased, he will complain of malaise, exhaustion, headache, sleeplessness, wandering pains, anorexia, nausea, and loss of weight. If these symptoms are disregarded, the injections continued, and the dose heedlessly increased, in time the patient's disease may take on an acute form. When intolerance manifests itself, whether by general fever reactions, by evidence of local reactions, or by some of the symptoms of constitutional impairment, the rule is never to inject while any of these symptoms are still present, but to wait until the temperature has returned to its usual height, until the cough and increased expectoration have lessened, and all evidence of constitutional impairment, such as anorexia, malaise, debility, etc., have disappeared. Indeed all evidences of intolerance must have been absent for at least two days before the injections are again taken up, then start with lower doses.

We have learned that "No reaction, no cure," has been a most misleading axiom, for we can have tuberculin immunity without reactions, and many reactions without any tuberculin immunity. Strong and frequent reactions are harmful, while patients who go through the entire treatment without appreciable fever reactions derive all the benefit that could be expected from the treatment.

Trudeau has formed his favorable impression of the influence of tuberculin by noticing how rarely the disease seemed to progress by the usual exacerbations and relapses in patients who were tolerating progressively increasing doses of tuberculin well, and in watching chronic cases, who were running a slow but steadily downward course in spite of the climatic and open-air treatment, derive marked benefit and even gradually return to apparent health after a full course of injections.

That tuberculin is not the vaunted and long-looked-for specific it was at first thought to be has been amply demonstrated by the bitter experience of the past. We have much to learn about tuberculosis, but even at the present state of our knowledge it seems established that the production of tuberculin immunity by the mild clinical

method is capable of favorably influencing the course of subacute and chronic tuberculosis, of prolonging life, and in many cases of aborting a commencing infection.

As to the type of cases suitable for tuberculin treatment, Denys and some of the Germans claim that even in acute cases good results may be occasionally expected by a careful course of injections.

Diagnostic Uses of Tuberculin.—The chief use to which Koch's original tuberculin has been put is as an aid to diagnosis in human beings and cattle, and for this purpose it has proved to be of inestimable value. Numerous experiments made by veterinary surgeons show that the injection of tuberculin in tuberculous cows in doses of 25 to 50 centigrams produces in at least 95 per cent. a rise of temperature of from 1° to 3° C. (2° to 5° F.). The febrile reaction occurs in from twelve to fifteen hours after the injection. Its intensity and duration do not entirely depend upon the extent of the tuberculous lesions, being even more marked when these are slight than in advanced cases. In non-tuberculous animals no reaction occurs, or one much less than in tuberculous animals, and the results obtained on autopsy justify the suspicion that tuberculosis exists if an elevation of temperature of a degree or more centigrade occurs and remains for ten hours from the subcutaneous injection of the dose mentioned. It must always be remembered that cattle may have a rise of temperature from other conditions, and it is only when due to tuberculin that infection is proved. When properly carried out, an error of more than 5 per cent. is impossible. For these injections, four-tenths c.c. of the original tuberculin is used, which for the convenience of administration is diluted with water.

United States Government Directions for Inspecting Herds for Tuberculosis.—"Inspections should be carried on while the herd is stabled. If it is necessary to stable animals under unusual conditions or among surroundings that make them uneasy and excited, the tuberculin test should be postponed until the cattle have become accustomed to the conditions they are subjected to, and then begin with a careful physical examination of each animal. This is essential, because in some severe cases of tuberculosis, on account of saturation with toxins, no reaction follows the injection of tuberculin, but experience has shown that these cases can be discovered by physical examination. This should include a careful examination of the udder and of the superficial lymphatic glands, and auscultation of the lungs.

"Each animal should be numbered or described in such a way that it can be recognized without difficulty. It is well to number the stalls with chalk and transfer these numbers to the temperature-sheet, so that the temperature of each animal can be recorded in its appropriate place without danger of confusion. The following procedure has been used extensively and has given excellent results:

"(*a*) Take the temperature of each animal to be tested at least twice, at intervals of three hours, before tuberculin is injected.

"(*b*) Inject in the evening, preferably between the hours of six and nine, $\frac{4}{10}$ c.c. of Koch's tuberculin previously diluted to 5 c.c. with sterile water. The injection should be made with a carefully sterilized hypodermic syringe. The most convenient point for injection is back of the left scapula. Prior to the injection the skin should be washed carefully with a 5 per cent. solution of carbolic acid or other antiseptic.

"(*c*) The temperature should be taken nine hours after the injection, and temperature measurements repeated at regular intervals of two or three hours until the sixteenth (eighteenth)[1] hour after the injection.

"(*d*) When there is no elevation of temperature at this time the examination may be discontinued; but if the temperature shows an upward tendency, measurements must be continued until a distinct reaction is recognized or until the temperature begins to fall.

"(*e*) If a cow is in a febrile condition tuberculin should not be used, because it would be impossible to determine whether, if a rise of temperature occurred, it was due to the tuberculin or to some transitory illness.

"(*f*) Cows should not be tested within a few days before or after calving, for experience has shown that the result at these times may be misleading.

"(*g*) In old, emaciated animals and in re-tests, use twice the usual dose of tuberculin, for these animals are less sensitive.

"(*h*) Condemned cattle must be removed from the herd and kept away from those that are healthy.

"(*i*) In making post-mortems the carcasses should be thoroughly inspected, and all the organs should be examined."

Diagnostic Use of Tuberculins in Man.—At first some believed that the irritation aroused in the tuberculous foci by the tuberculin sometimes caused a dissemination of the bacilli and an increase in the disease. When carefully used, however, in suitable cases there is probably no danger. A drawback to its usefulness is that it does not reveal the extent of the disease, nor whether the tuberculosis is active. It is, however, of great value in selected cases, both surgical and medical, where slight tuberculosis is suspected, and yet no decision can be reached. In the small first dose advised an absolutely latent infection would usually give no rise of temperature. I quote here Dr. Trudeau upon the use of the test:

"The range of the patient's temperature is ascertained by taking it at 8 A. M., 3 P. M., and 8 P. M., for three or four days before making the test. The first injection should not exceed 0.5 mg. in adults and 0.3 in small children, and if any fever is habitually present should be even less, and is best given early in the morning or late at night, as the

[1] The directions allow temperatures to be stopped the sixteenth hour, but even when there is no reaction at all it is much safer to always take temperatures for eighteen hours. We have found now and then a tuberculous cow that reacted on the eighteenth hour for the first time.

typical reaction usually begins, in my experience, within six or twelve hours. Such a small dose, while it will often be sufficient to produce the looked-for rise of temperature, has, under my observation, never produced unpleasant or violent symptoms. An interval of two or three days should be allowed between each of the two or three subsequent injections it may be necessary to give, as reaction in very rare cases may be delayed for twenty-four or even thirty-six hours. On the third day a second dose of 1 mg. is given, and if no effect is produced a third, of 2 mg., three days later. In the great majority of cases of latent tuberculosis an appreciable reaction will be produced by the time a dose of 2 mg. has been reached. If no effect has been caused by the tests applied as above I have usually gone no farther, and concluded that no tuberculous process was present, or at least not to a degree which need be taken into account in advising the patient, or which would warrant insisting on a radical change in his sourroundings and mode of life. If some slight symptoms, however, have been produced by a dose of 2 mg., it may be necessary to give a fourth injection of 3 mg. in order to reach a positive conclusion. Nevertheless, it should be borne in mind that in a few cases the exhibition of even larger doses may cause reaction, when the smaller do not, and indicate the existence of some slight latent tuberculous lesion, and the negative result should not, when applied within the moderate doses described, be considered absolutely infallible."

"No evidence in connection with the tuberculin test as applied to man and animals has been forthcoming thus far from those who have made use of it, which would tend to sustain the general impression that this method is necessarily dangerous and tends invariably to aggravate the disease, and my own experience has developed nothing which would seem to confirm this impression. It is evident that the size of the doses given has much to do with the limitations of this method for usefulness, and the correctness of the conclusions reached by its application. The tuberculin used is also a matter of some importance in determining the dosage, as different samples vary considerably in their efficiency. If the test be pushed to the injection of such large amounts at 10 mg. or more, as advocated by Maragliano, such doses are by no means free from the objection of occasionally causing unpleasant and sometimes dangerous symptoms; and even if the amount given be not carried to the dose of 10 mg., which is known to produce fever in healthy subjects, it is likely that on account of individual susceptibility or the presence of some other morbid process in the body, reaction will be found to occur with the larger doses when no tuberculous process exists. The adoption of an initial dose so small as to guard against the absolute possibility of producing violent reactionary symptoms, and the graded increase of the subsequent doses within such quantities as are known never to produce reaction in healthy individuals, would seem to afford the best protection against unpleasant results and misleading evidence."

Von Pirquet's Cutaneous Tuberculin Test.—This has for many

purposes supplanted the subcutaneous injections. It is perfectly harmless. This is carried out by placing a drop of a 25 or a 50 or a 100 per cent. solution of tuberculin upon the skin of the forearm and then with a needle or instrument making through it a slight abrasion without drawing blood, as in vaccination. The skin is abraded at another point without the tuberculin as a control. Within 12 to 24 hours a papule with a surrounding congested area forms about the inoculated point much as appears after the use of cowpox vaccine in a previously vaccinated person. The test is frequently carried out by making a scratch about an inch in length. This should if possible not cause bleeding. The tuberculin on a probe or slip of wood is rubbed into the scratch. Sometimes one spot is tested with the 10 per cent. solution and a second with the 25 per cent. A reaction with the weaker solution is believed to indicate some activity in the process, while the stronger may give a reaction in a person having a recently healed lesion.

Moro's Test.—Equal parts of tuberculin and lanolin are mixed together to make an ointment. A little of this is rubbed thoroughly upon a portion of the skin of the arm. Twelve to twenty-four hours afterward a crop of papules develops in cases in which the cutaneous tests proves effective.

Directions for the Ophthalmo-tuberculin Diagnostic Test.—Method of Application.—Two solutions in two strengths are employed in diagnosis, one of the alcohol precipitate in 0.5 per cent. and 1 per cent. and the other of 1 and 2 per cent. of tuberculin (T. O.). The weaker and stronger may be used successively in each eye if time permits. In this way unnecessarily severe reactions may be avoided.

The eyelid should be held down until the drop is distributed about the sac without overflowing on the cheek. The same eye should not be used for a second test as it usually becomes sensitized to some degree by one test. The tested eye should be kept from external irritation due to rubbing, wind, dust, and smoke.

Reactions.—The first symptoms of a reaction appear in from 3 to 12 hours in most cases, but may be delayed 24 and even 48 hours, and continue for a week. The presence of a reaction is indicated by a scratchy feeling, secretion and redness of the inner canthus, caruncle or lower lid which may increase and include the entire conjunctiva with œdema of lids.

Schema for Recording Reactions.—The following schema is propose for recording the degree of reaction.

Negative: No difference in color when lower eyelids are pulled down.

Doubtful: Slight difference with redness of caruncle.

\+ = Distinct palpebral redness with secretion.

\+ + = Ocular and palpebral redness with secretion well marked.

\+ + + = Deep injection of entire conjunctiva with œdema of lids and photophobia, and secretion.

Contraindications.—Any existing disease of either eye or lids.

Interpretation of Reaction.—This is practically the same as with the cutaneous test. About 80 per cent. of latent or active tuberculosis react and about 40 per cent. of very advanced cases. Persons very ill from other diseases frequently do not react. Tuberculosis so slight that it is impossible to detect it during life may give a good reaction. A negative result in a person in fair general health indicates strongly that no tuberculosis is present. The location of the tuberculosis is of course not revealed by the test.

Deleterious Effects.—Our personal experience accords with that published by others that about once in every four hundred tests a serious conjunctivitis, keratitis, or iritis results. This possible injury has led largely to giving preference to the cutaneous test.

Antituberculous Serum.—Every conceivable way of obtaining the true products of the tubercle bacilli has been tried, so as to cause the injected animals to produce antibodies both antitoxic and bactericidal. At present Maragliano and Marmorek are presenting claims that their sera are truly curative. Although both these men have had a large experience in this field of investigation, it is probable that the final judgment will be that little good comes from the injection of their serum. Very few observers have succeeded in obtaining appreciable results with the serums prepared by other experimenters. In spite of much conflicting testimony, it is probably safe to assert that no sera now obtainable have any great value. Nor as we look at the progressive nature of tuberculosis can we see much ground to hope for the abundant development of curative substances in the blood of animals. This view, however, in no way lessens the necessity of continued endeavor until every method conceivable has been tried.

Prophylaxis.—Meanwhile all energies should be directed to the prevention of tuberculosis, not only by the enforcement of proper sanitary regulations as regards the care of sputum, milk, meat, disinfection, etc., but also by continued experimental work and by the establishment of free consumptive hospitals, and by efforts to improve the character of the food, dwellings, and conditions of the people in general, we should endeavor to build up the individual resistance to the disease. It may be years before the public are sufficiently educated to coöperate with the sanitary authorities in adopting the necessary hygienic measures to stamp out tuberculosis entirely; but, judging from the results which have already been obtained in reducing the mortality from this dread disease, we have reason to believe that in time it can be completely controlled.

Among the numerous medical agents that have been tried without avail to protect animals against the action of the tubercle bacillus may be mentioned tannin, menthol, sulphuretted hydrogen, mercuric chloride, creosote, creolin, phenol, arsenic, eucalyptol, etc.

Agglutination.—The results obtained by various observers have been very conflicting. Two methods are employed in making the test. In one a vigorous growth of bacilli is dried, ground up, and an emulsion made. In the other Arloing and Courmont grow the cul-

ture for a time on potato and then in bouillon. In this way a homogeneous culture of separate bacilli is obtained which can be used for agglutination. The examination is usually made macroscopically, and requires twelve to twenty-four hours. At present the test cannot be advised as useful in diagnosis as the sera of cases suffering from tuberculosis frequently fail to give a reaction, while the sera from those having no detectable tuberculosis frequently cause a good reaction. A positive agglutination test is thought by some to be a favorable sign as indicating resistance to infection by the body. A reaction in dilutions of 1:10 or 1:15 is considered a positive test.

The Tubercle Bacillus of Cattle, Pigs and Sheep, and its Relation to Human Tuberculosis.—Among the domestic animals tuberculosis is most common in cattle. On account of the milk which they provide for our use, and which is liable to contain bacilli, the relation of these to human tuberculosis is a matter of extreme importance.

The chief seat of the lesions is apt to be the lungs, and with them the pleura; less often the abdominal organs and the udder are affected. In pigs and horses the abdominal organs are most often involved, then the lungs and lymphatic glands. In sheep and goats tuberculosis is rare.

Differences between Tubercle Bacilli of Human and Bovine Type.—As has been already noted in the tables given of the incidence of bovine and human infection, it is possible to tell in any case the type of infection. The essential differences are in cultural characteristics and in virulence for rabbits and calves.

Cultural Differences.—The bovine bacillus grows very poorly when isolated, the human bacillus very freely. This is noted on plain egg, but to a less extent than on glycerin egg. The glycerin restrains or adds little to the growth of bovine bacilli, but increases markedly the amount of growth of the human bacillus. In fact, primary cultures on glycerin egg of bovine material commonly fail. This difference is very noticeable in the first few generations and is sufficient in the great majority of instances for differentiation to one who has had some experience with such cultures. Further, the majority of human strains can be transplanted to glycerin potato or glycerin broth and give vigorous growth in the first few generations, whereas the bovine bacillus fails or growth is very slight. After further cultivation the bovine bacillus gradually increases its amount of growth until it is indistinguishable from the human type. This increase in luxuriance may be rapid or very slow.

Rabbit Virulence.—The bovine bacillus is exceedingly virulent for rabbits by any method of inoculation; the human bacillus only slightly so. The best method of differentiation is by intravenous inoculation. A small amount of culture is weighed after the moisture has been extracted with filter-paper and a suspension made in normal saline and diluted so that 1 c.c. $= \frac{1}{100}$ mg. of culture; this amount is then injected into the ear vein of a rabbit. If the rabbit survives for from forty to fifty days, and on autopsy shows only lesions in the

lungs or kidneys or both, the strain is of the human type. With the bovine type of bacillus the rabbit will die in the majority of instances before or about this time, if not it may be killed. On autopsy a progressive generalized tuberculosis will be found. The lesions in the lungs will be very marked, the tubercles having become confluent with caseous centres. The liver or spleen or both will be peppered with tubercles. Tubercles will be present in the great majority of cases in the superficial lymph nodes and also in those of abdomen and thorax. There may be tubercles on the heart, in the rib marrow, or over the peritoneum.

These two differences alone are sufficient to differentiate in every case the type of bacillus. It must be insisted upon again that the cultural characteristics be observed in the early generation and further that the virulence be tested in early generations. In case the bovine culture does not afford sufficient material for weighing, a suspension can be made and compared with a weighed suspension.

Virulence for Calves.—In proving the non-identity of the two bacilli, calf experiments were resorted to. This was necessary as the supposed bovine cultures from children would have to be virulent for calves to the same extent as cultures from bovine material. The commonly used method was the subcutaneous inoculation in the side of the neck with 50 mg. of culture. The human type of bacillus caused only a local lesion or at most a spreading to the nearest lymph node. The bovine bacillus, on the other hand, caused a generalized tuberculosis which was or was not fatal. Sufficient data has been accumulated to make this test practically unnecessary for the determination of type.

Differences in Morphology.—The bovine bacillus tends to be shorter, thicker and solidly stained; the human type tends to be longer, slimmer, usually bent, and shows beading and irregularities in staining. We have found this difference most marked on glycerin egg, slight or imperceptible on other media.

Besides the above differences Theobald Smith made the interesting discovery that the production of acid differed with the two types when grown on glycerin broth. The bovine type renders the bouillon less and less acid; this may even progress till the medium becomes slightly alkaline to phenolphthalein. The human type causes a preliminary fall in the acidity; as growth progresses the acidity is then gradually increased, and may exceed the original acidity of the broth used. This difference is evident in tuberculin made from the two types of bacilli. The bovine tuberculin is alkaline or very slightly acid while human tuberculin is markedly acid. The change is only noticed when glycerin is used in the media. Whether this difference is specific is doubtful. The work of more recent investigators would seem to show that this difference, like all differences between the types, is purely quantitative, and that different strains vary in their reactions and give intermediate reactions between these two extremes.

Bird (Avian) Tuberculosis.—Tuberculosis is very common and

infectious among fowl. The bacillus grows easily and freely on glycerin media. It tends to form a moist or even slimy growth, and commonly produces an orange pigment. It is able to grow at a higher temperature than mammalian tubercle bacilli, the latter failing to grow above 41° C.; the former growing at even higher temperatures. Guinea-pigs are less susceptible to inoculation with avian tubercle bacilli, and the virulence for these animals is usually quickly lost. Rabbits are somewhat more susceptible. Rats and mice are spontaneously infected with avian tubercle bacilli and are supposed to be an important factor in spreading the disease. Birds are refractory, with few exceptions, to infection with the mammalian tubercle bacillus. Parrots, however, are susceptible to infection with all three types and commonly have spontaneous tuberculosis caused by the human type of bacillus.

Stability of the Different Types of Bacilli.—The fact that the agglutination reactions and the tuberculin reactions of the different types is similar shows their close relationship. This has led to the endeavor to change one type into the other. This is usually done by passage through animals. The results have been peculiar. Some cultures have been passed through a series of calves without any change except for a moderate increase in virulence. Other cultures seem to have completely changed their type. We believe that this is not a change of type, but an additional bovine infection. Strong negative evidence is the fact that the bovine bacillus when infecting man loses none of its characteristics, though present in the human body for years.

Tuberculosis in Fish.—In certain species of fish a tuberculous disease has been noted. The bacilli have the staining characteristics of the warm-blood types, but do not grow at body temperature and do not affect mammals.

Methods of Examination for Tubercle Bacilli and Other Associated Bacteria.—One of the most important results of the discovery of the tubercle bacillus relates to the practical diagnosis of tuberculosis. The staining peculiarities of this bacillus renders it possible by the bacteriological examination of microscopic preparations to make an almost absolutely positive diagnosis in the majority of cases. A still more certain test in doubtful cases is the subcutaneous or intraperitoneal injection of guinea-pigs, which permits of the determination of the presence of numbers of bacilli, so small as to escape detection by microscopic examination. For the animal test, however, time is required—at least three weeks, and, when the result is negative, at least six weeks—before any positive conclusion can be reached, for when only a few bacilli are present tuberculosis develops slowly in animals. In disinfection experiments where many dead bacilli are injected, care must be taken to exclude the local effect of dead bacilli. In doubtful cases a second guinea-pig should be injected from the first.

Microscopic Examination of Sputum for the Presence of Tubercle Bacilli. 1. **Collection of Material.**—The sputum should be collected in a clean bottle (two-ounce) with a wide mouth and a water-tight stopper, and the bottle labelled with the name of the patient or with

some other distinguishing mark. The expectoration discharged in the morning is to be preferred, especially in recent cases, and the material should be coughed up from the lungs. Care should be taken that the contents of the stomach, nasopharyngeal mucus, etc., are not discharged during the act of expectoration and collected instead of pulmonary sputum. If the expectoration be scanty the entire amount discharged in twenty-four hours should be collected. In pulmonary tuberculosis the purulent, cheesy, and mucopurulent sputum usually contains bacilli; while pure mucus, blood, and saliva, as a rule, do not. When hemorrhage has occurrred, if possible, some purulent, cheesy, or mucopurulent sputum should be collected for examination. The sputum should not be kept any longer than necessary before examination, for, though a slight delay or even till putrefaction begins, does not vitiate the results so far as the examination for tubercle bacilli is concerned, it almost destroys any proper investigation of the mixed infection present; it is best, therefore, to examine it in as fresh a condition as possible, and it should be kept on ice until examined if cultures are to be made.

2. **Methods of Examination.**—*Examination for Tubercle Bacilli.*—Pour the specimen into a clean, shallow vessel, having a blackened bottom—a Petri dish placed upon a sheet of dull black paper answers the purpose—and select from the sputum some of the true expectoration, containing, if possible, one of the small white or yellowish-white cheesy masses or "balls." From this make rather thick cover-glass or slide "smears" in the usual way. In doubtful cases a number of these coarse or fine particles should be placed on the slide. The material being thick, should be evenly spread and very thoroughly dried in the air before heating. Immerse this in a solution of Ehrlich's aniline-water fuchsin or better in the Ziehl-Neelson carbol-fuchsin solution contained in a thin watch-glass or porcelain dish, or hold slide completely covered with solution in the Cornet forceps and steam over a small flame for two minutes. Then remove and wash with water. Now decolorize by immersing the stained preparation in a 3 per cent. hydrochloric acid solution in alcohol for from one-half up to one minute, removing at the time when all color is just about gone from the smear. Wash thoroughly with water, and make a contrast stain by applying a cold solution of Loeffler's alkaline methylene blue—

Concentrated alcoholic solution of methylene blue	30 c.c.
Caustic potash (1:10,000 solution)	100 c.c.

for from fifteen to thirty seconds. Wash with water; press between folds of filter-paper; dry in air; mount, and examine.

The tubercle bacilli are distinguished by the fact that they retain the red color imparted to them in the fuchsin solution, while the other bacteria present, having been decolorized in the acid solution, take the contrast stain and appear blue. (See Plate I., Figs. 1 and 2.)

Various methods have been suggested for the staining of tubercle

bacilli, but the original method, as employed by Koch, or some slight modification of it, is so satisfactory in its results that it is still generally employed. The above is a slight modification of the Koch-Ehrlich method, differing from it chiefly in the use of a weak for a strong acid decolorizer. It has been found that the strong acid solution originally employed (5 per cent. sulphuric acid solution in alcohol) often decolorizes some of the bacilli entirely by its too energetic action, and that a weaker decolorizer, such as the above, gives more uniform results.

The Koch-Ehrlich aniline-water solution decomposes after having been made for a time, so that it must be freshly prepared as needed. Solutions older than fourteen days should not be used. The advantages in using Ziehl's carbol-fuchsin solution are that it keeps well and is more convenient for use in small quantities.

Another method, which is often of value on account of its simplicity and rapidity of performance, is that of Fränkel as modified by Gabbett. This consists in staining the "smear" with steaming Ziehl's carbol-fuchsin solution for from one to two minutes, and then, after washing in water, placing it from one-half to one minute directly in a second solution which contains both the acid for decolorizing and the contrast stain. This second solution consists of

Sulphuric acid	25 c.c.
Methylene blue in substance	2 grm.
Water	75 c.c.

It is then washed with water and is ready for examination. The tubercle bacilli will remain red as stained by the fuchsin, while all the other bacteria will be tinted blue.

When the number of tubercle bacilli in sputum is very small they may easily escape detection. Methods have, therefore, been suggested for finding them under these circumstances. Several stains have been advised in this case, the simplest and most satisfactory being that of Herman. The advantage is that more bacilli are stained than with carbol-fuchsin. The stain consists of A, crystal violet, 3 per cent. alcoholic solution, B, ammonium carbonate, 1 per cent. in distilled water. Mix 1 part of A with 3 parts of B just before using. Steam as with carbol-fuchsin, decolorize in 10 per cent. nitric acid, rinse for a few seconds in alcohol, wash, and counterstain with Bismark brown. The tubercle bacilli are stained violet. Where the slides stained with carbol fuchsin are negative, this stain will occasionally demonstrate bacilli.

Biedert advises the following method: Dilute 10 c.c. of sputum with 90 c.c. of water. Heat over the flame and add a 10 per cent. solution of sodium hydroxide, stirring, till the mucus is dissolved. Allow the coarser particles to separate. Add a few drops of phenolphthalein solution and neutralize with dilute acetic acid. Pour into twice its bulk of alcohol and allow to sediment. The coagulum that forms collects all the tubercle bacilli. Concentrate the sediment by centrifuging and make smears. Fix to the slide if necessary with some of the patient's own sputum.

Uhlenhuth advises the use of antiformin. This is a patented preparation consisting of a mixture of sodium hydroxide and sodium hypochlorite solution. If this is mixed with sputum so that the total strength is about 15 per cent. of antiformin, the sputum quickly becomes fluid. This should be thinned with water or alcohol to help reduce the specific gravity of the mixture and centrifuged. The sediment is then mixed with water and recentrifuged, and the washed sediment used for smears. Besides the dissolving action, antiformin kills most of the bacteria in the sputum, but not the tubercle bacilli, though they are slowly affected, so that sediment may be used for cultural purposes or injection into guinea-pigs.

A comparison of the above methods made by us gave the following results. Of twenty-eight sputa negative with carbol-fuchsin, two showed bacilli after a few minutes search with the crystal violet stain. On restaining with carbol-fuchsin and giving only a light counterstain with methylene blue the negative slides were also positive. Of the remaining twenty-six, four (15 per cent.) were quickly positive in the antiformin sediment when stained with crystal violet, whereas only three were positive with carbol-fuchsin and only after restaining as above. It is advisable, therefore, in using carbol-fuchsin to have only a light counterstain to make the method most efficient, and control the results with crystal violet if negative.

Detection of Tubercle Bacilli in Urine and Fæces, Etc.—The catheterized urine is centrifuged. If little sediment appears, the upper portion of the fluid is removed and more urine added and again centrifuged. If the urine is rich in salts of uric acid, the same may be diminished by carefully warming the urine before treating it. If too alkaline add a little acetic acid.

The fæces are examined for any purulent or mucous particles. If none are found, larger masses of fæces are removed and then the rest diluted and centrifugalized. The examiner must remember that bacilli swallowed with the sputum may appear in the fæces. In examining cerebrospinal fluid for tubercle bacilli it must be remembered that the majority of the bacilli are entangled in the delicate clot that forms. This is also the case in other serous fluids, but in ascitic or pleuritic fluid they are usually very few in number. A method has been devised called inoscopy to render tubercle bacilli easier of detection in serous fluids. The fibrinous clot which forms is freed from the rest of the fluid and treated with about 30 c.c. of the following digestive mixture:

Pepsin	2 grms.
Glycerin	10 c.c.
Hydrochloric acid (sp. gr. 1.18)	10 c.c.
Sodium fluoride	3 grms.
Distilled water	1000 c.c.

The mixture is incubated twenty-four hours, and when digested and homogeneous, centrifuged and smears made from the sediments, using egg albumin to fix it to the slide.

Examination for Other Bacteria (Mixed Infection).—With regard

to the *bacteriological diagnosis* of pulmonary phthisis, many consider that it is not enough to show only the presence of tubercle bacilli; it is held to be of importance, both for purposes of prognosis and treatment, that the presence of other microörganisms which may be associated with the tubercle bacillus should also be determined. It is now usual to distinguish pure tuberculosis of the lungs from a mixed infection. Phthisis due to the tubercle bacillus alone, which constitutes but a small percentage of all cases, may occur almost without febrile reaction; or when fever occurs the prognosis is unfavorable, thus indicating that the disease is already advanced. It is in the uncomplicated forms of phthisis, moreover, where one must expect if anywhere the best results from treatment with tuberculin or antituberculous serum. The majority of cases, however, of pulmonary tuberculosis show a mixed infection, especially with varieties of the streptococcus and pneumococcus. These cases may be active, with fever, or passive, without fever, according, perhaps, as the parenchyma of the lung is invaded by the bacteria; or they are only superficially located in cavities, bronchi, etc. Mixed infection with the staphylococcus, other micrococci, and with the influenza bacilli have also been frequently met with by us. The tetragenus has not been often detected by us in thoroughly washed fresh sputum. At present the facts seem to prove that the tubercle bacilli have in the great majority of cases, at least shortly before death, a much more important rôle than the associated bacteria.

Sputum Washing.—Some of the associated bacteria found in the expectoration come from the diseased areas of the lungs, while others are merely added to the sputa as it passes through the mouth or are developed after gathering. To endeavor to separate the one from the other we wash the sputa. The first essential is that the material is to be washed within a few minutes, and certainly within an hour after being expectorated. If a longer time is allowed to intervene, the bacteria from the mouth will penetrate into the interior of the mucus, and thus appear as if they came from the lungs. Sputum treated twenty-four hours after its expectoration is useless for examining for anything except the tubercle bacillus. A rough method is to pour some of the specimen of sputum to be examined into a convenient receptacle containing sterile water, and withdraw, by means of a sterilized platinum wire, one of the cheesy masses or thick "balls" of mucus. Pass this loop five times through sterile water in a dish; repeat the operation in fresh water in a second and third dish. Spread what remains of the mass on cover-glasses and make smear preparations; stain and examine. With another mass inoculate ascitic bouillon in tubes and agar in plates.

When we wish thoroughly to exclude mouth bacteria, a lump of the sputum raised by a natural cough is seized by the forceps and transferred to a bottle of sterile water and thoroughly shaken; it is then removed to a second bottle of bouillon and again thoroughly shaken. From this it is passed in the same way through four other bottles of

bouillon. A portion of the mass is now smeared over cover-glasses, and the rest inoculated in suitable media, such as agar in Petri dishes, and ascitic fluid bouillon in tubes. If desired, the bacteria washed off in the different washings are allowed to develop.

Practical Notes on the Examination for Mixed Infection.—1. The difficulties to be overcome, in order to obtain sputum consisting presumably of exudate from the deeper portions of the lungs, are so great that the collection of the specimens should be supervised by the bacteriologist in charge of the work of examination.

2. Specimens of sputum collected even with the greatest precaution may give evidence of decided mouth infection unless immediately washed.

3. The sputum must be examined very soon after collection.

4. The culture medium used for the final cultures must be suitable for the growth of the microörganisms.

5. At least two successive examinations of sputum should be made in each case.

6. The results, especially as to the number of colonies, vary according to the size and tenacity of the ball of sputum washed—*e. g.*, a small ball of sputum which becomes more or less broken up upon thorough shaking may contain very few or no bacteria.

Williams, in the examination of the sputum in some 40 cases, came to the following conclusions: 1. The presence of a large number of bacteria in a satisfactory and thoroughly washed specimen of sputum indicates that these bacteria probably play an active part in the disease. 2. The presence of a small number of bacteria in such sputum does not necessarily indicate that they are not active in that case, for they may penetrate more or less deeply into the lung tissue, and produce pathological changes without being thrown off in large numbers with the exudate. It is probable, however, that, as a rule, the smaller the number found the less the degree of mixed infection.

3. Cases of clinically secondary infection frequently give pure cultures of some one organism (pneumococcus, influenza bacillus, or streptococcus), which are capable of causing the symptoms.

4. In the majority of severe cases of clinically mixed infection many organisms have been found which usually have belonged to several different species or varieties.

5. In the majority of cases of clinically non-mixed infection very few organisms have been found.

6. Only bacteria which might cause pathological changes were present.

7. Very few of the organisms found were markedly virulent in rabbits, even though coming from severe cases of mixed infection.

The virulence for laboratory animals of bacteria obtained from the sputum is, therefore, no indication of their virulence for man because of the impossibility of reproducing in such animals the exact condition of susceptibility present in human infection.

General Rules in Microscopic Examination of Sputum.—Always

make two smear preparations from each specimen. Report no result as negative until at least two preparations have been subjected to a thorough search with a $\frac{1}{12}$ oil-immersion or 2 mm. apochromatic lens by means of a mechanical stage. From a very large experience in the examination of sputum for tubercle bacilli, the New York Health Department bacteriologists have concluded that the examination of two preparations of each specimen, in the careful manner described above, is usually sufficient to demonstrate the presence of the bacilli when they are present in the sputa, and they are usually found to be present to this extent in fairly well-developed cases of pulmonary tuberculosis, and in many cases which are in the incipient stage. There are, however, undoubted cases of incipient pulmonary tuberculosis which require the examination of many preparations before the tubercle bacillus can be found; and cases also occur in which the sputum for a time does not contain the bacilli, which were, nevertheless, present at an earlier period, and which again appear later. Therefore, if cases occur which may be still regarded as possibly tuberculosis, further examinations of the sputum should be made. It should also be constantly borne in mind that the demonstration of the presence of tubercle bacilli in the sputum prove about as conclusively as anything can the existence of some degree of tuberculosis; but that the absence of tubercle bacilli or the failure to find them microscopically does not positively exclude the existence of the disease. Here tuberculin can be made use of.

Staining of Tubercle Bacilli in Tissues.—Thin sections of tuberculous tissues may be stained by the same methods recommended for cover-glass preparations, except that it is best not to employ heat to any extent. Fixation in bichloride of mercury is better than in alcohol. Formalin is a bad fixative, as it makes the tissues hold the fuchsin with as much tenacity as the bacilli. Both paraffin and celloidin may be used for embedding; but the former is better.

EHRLICH'S METHOD.—Place the paraffin sections in aniline fuchsin and leave at 37° C. for from six to twelve hours, or at about 80° C. for three to five minutes, the sections are then washed in water; then decolorize by placing them for about half a minute in dilute nitric acid (10 per cent.), or in 3 per cent. hydrochloric acid in alcohol; wash in 60 per cent. alcohol until no more color is given off; counterstain for two or three minutes in a saturated aqueous solution of methylene blue, or, better, with hæmatoxylin; wash in water; dehydrate with absolute alcohol; clear in oil of cedar or xylol, and mount in xylol balsam.

METHOD OF ZIEHL-NEELSON.—Stain the section in warmed carbol-fuchsin solution for one hour; the temperature to be not over 45° to 50° C. Decolorize for a few seconds in 5 per cent. sulphuric acid, then in 70 per cent. alcohol, and from this on as in the Ehrlich method.

Inoculation of Animals.—The inoculation of suspected material into guinea-pigs sometimes produces tuberculosis when no bacilli could be detected by microscopic examination. The material should be injected subcutaneously as already described (p. 20).

Cultivation.—This is so difficult and requires so much time that it is not used except in important investigations upon the nature of the tubercle bacilli. The special methods have already been given.

CHAPTER XXIV.

BACILLI SHOWING STAINING REACTIONS SIMILAR TO THOSE OF THE TUBERCLE BACILLI—LUSTGARTEN'S BACILLUS—SMEGMA BACILLUS—LEPROSY BACILLUS—GRASS BACILLI.

LUSTGARTEN'S BACILLUS—SMEGMA BACILLUS.

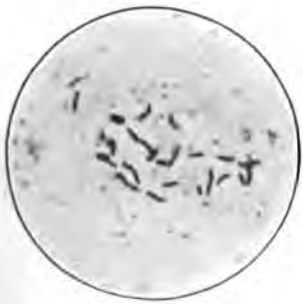
Bacilli were discovered by Lustgarten in syphilitic lesions or syphilitic ulcers (1884), and believed by him to be the specific cause of this disease. It has since been shown that in normal smegma from the prepuce or the vulva bacilli are found in great abundance, similar in their morphology to the bacillus of Lustgarten, but differing, as a rule, slightly in certain staining peculiarities. (See Fig. 111.)

Morphology.—Straight or curved bacilli, which bear considerable resemblance to tubercle bacilli, but differ from them in staining reactions. The bacilli are not usually found free in the tissues, but commonly lie singly or sometimes in groups within the interior of cells, having a round, oval, or polygonal form, and apparently somewhat swollen.

Staining.—The bacillus of Lustgarten *stains* with almost as much difficulty as the tubercle bacillus, but is much less resistant to the action of certain decolorizing agents, such as mineral acids, particularly sulphuric acid.

Biological and Pathogenic Properties.—Numerous attempts have been made to cultivate the bacillus of Lustgarten on artificial media, but with doubtful success. The inoculation of animals has also given only negative results.

Fig. 111



Smegma bacilli, similar in characteristics to Lustgarten's bacillus. ×1100 diameters.

Lustgarten's bacillus has been found in various syphilitic tissues and lesions, in beginning sclerosis, in the papules, in condylomata and gummata, and not only in the vicinity of the genitals, but also in the mouth, throat, heart, and brain. No satisfactory experimental evidence has been given of its causative relation to syphilis. The finding of saprophytic bacilli—the so-called smegma bacilli—(Fig. 111 and Plate I., Fig. 4) almost identical morphologically with the bacillus of Lustgarten, under the prepuce of healthy persons, does not prove the identity of the two bacilli, though, in the absence of cultures and inoculation experiments,

we have not the means of establishing their relationship to one another. The smegma bacilli have never been identified in other parts of the body, except in the neighborhood of the genitals. While the bacillus of Lustgarten cannot resist the prolonged decolorizing action of acids, but is resistant to the action of alcohol, the smegma bacillus, when stained, is rather quickly decolorized by alcohol, but quite resistant to 5 per cent. sulphuric acid solution. Besides, Lustgarten's bacillus has been found in papules, in gummata, and other syphilomata, where there seems no probability whatever of the smegma bacillus having emigrated.

The *differential diagnosis* of Lustgarten's bacillus must be made from the tubercle bacillus, the smegma bacillus, and the leprosy bacillus. According to Hueppe, the differential diagnosis between these four organisms depends upon the following reactions: When stained by the carbol-fuchsin method, commonly employed in staining the tubercle bacillus, the syphilis bacillus becomes almost instantly decolorized by treatment with mineral acids, particularly sulphuric acid; whereas, the smegma bacillus resists such treatment for a much longer time, and the lepra and tubercle bacilli for a still longer time. On the other hand, if decolorization is practised with alcohol instead of acids, the smegma bacillus is the first to lose its color. The bacillus tuberculosis and the bacillus of leprosy are both very retentive of their color, even after treatment with acids and alcohol. If, then, we treat the preparation, stained with carbol-fuchsin, with sulphuric acid, the syphilis bacillus becomes almost at once decolorized. If it is not immediately decolorized, treat with alcohol; if it is then decolorized, it is probably the smegma bacillus. If it is still not decolorized, and it lies between these four bacilli only, it is either the leprosy or the tubercle bacillus.

By these methods the differential diagnosis can usually be made. In all investigations of importance, however, animal inoculations should also be made, as by this means alone can a positive diagnosis from tuberculosis be established. Especial care should be observed in the examination of syphilitic ulcers of the genital region, as in this situation the smegma bacilli are almost always present.

LEPROSY BACILLUS—B. LEPRÆ.

The bacillus of leprosy was discovered by Hansen and Neisser (1879) in the leprous tubercles of persons afflicted with the disease. This discovery was confirmed by many subsequent observers.

Morphology.—Small, slender rods resembling the tubercle bacilli in form, but somewhat shorter and not so frequently curved. The rods have pointed ends, and in stained preparations unstained spaces, similar to those observed in the tubercle bacillus, are seen. They *stain* readily with the aniline colors and also by Gram's method. Although differing slightly from the tubercle bacillus in the ease with which they take up the ordinary aniline dyes, they behave like tubercle

bacilli in retaining their color when subsequently treated with strong solutions of the mineral acids and alcohol. The slight difference in staining characteristics is too little to be relied upon for diagnostic purposes.

Biological Characters.—Clegg[1] reported in 1908 that he had been able to cultivate an acid-fast bacillus from cases of leprosy in symbiosis with amœbæ and cholera vibria. By heating a symbiotic culture the leprosy bacillus was obtained in pure culture. From the first cultures different media were successfully inoculated. On nutrient agar the surface colonies are small and brownish. Blood serum is liquefied after ten days. Lactose is not fermented.

FIG. 112

Leprosy bacilli in nodule. (Kolle and Wassermann.)

Pathogenesis.—Numerous inoculation experiments have been made on animals with portions of leprous tubercles, but there is no conclusive evidence that leprosy can be transmitted to the lower animals by inoculation. The inference that this bacillus bears an etiological relation to the disease with which it is associated is based chiefly upon the demonstration of its constant presence in leprous tissues (Fig. 112). Subcutaneous inoculations of cultures in guinea-pigs have produced local lesions which resemble leprous lesions in man. This has been repeated by Duval who states that he was able to continue the growth on later transfers.

The bacilli are found in all the diseased parts, and usually in large numbers, especially in tubercles on the skin, in the conjunctiva and cornea, the mucous membranes of the mouth, gums, and larynx, and in the interstitial processes of the nerves, testicles, spleen, liver, and kidneys. The rods lie almost exclusively within the peculiar round or oval cells of the granulation tissue which composes the leprous tubercles, either irregularly scattered or arranged parallel to one another. In old centres of infection the leprosy cells containing

[1] The Philippine, Jour. of Science, Vol. iv, No. 6.

the bacilli are larger and often polynuclear. Giant cells, such as are found in tuberculosis, are claimed to have been observed by a few investigators (Boinet and Borrel). In the interior of the skin tubercles, the hair follicles, sebaceous and sweat-glands are often attacked, and bacilli have sometimes been found in these (Unna, etc.). Quite young eruptions often contain a few bacilli. A true caseation of the tubercles does not occur, but ulceration results. During acute exacerbations with development of new lesions bacilli have been observed in the blood.

In the anæsthetic forms of leprosy the bacilli are found most commonly in the nerves and less frequently in the skin. They have been demonstrated in the sympathetic nervous system, in the spinal cord, and in the brain. The *Bacillus leprœ* occurs also in the blood, partly free and partly within the leukocytes, especially during the febrile stage which precedes the breaking out of fresh tubercles (Walters and Doutrelepont). The bacilli have also been found in the intestines, in the lungs, and in the sputum, but not in the urine.

With regard to the question of the direct inheritance of the disease from the mother to the unborn child there is considerable difference of opinion. Some cases have been reported, however, in which a direct transmission of the bacillus during intrauterine life seems to be the only or most plausible explanation of the infection. At the same time, we have no positive experimental evidence to prove that such an infection does take place. Although many attempts have been made to infect healthy individuals with material containing the bacilli of leprosy, the results are not conclusive. Even the experiments made by Arning, who successfully infected a condemned criminal in the Sandwich Islands with fresh leprous tubercles, and which have been regarded as positive evidence of the transmissibility of the disease in this way, are by no means conclusive; for, according to Swift, the man had other opportunities for becoming infected. These negative results, together with the fact that infection does not more frequently occur in persons exposed to the disease, may possibly be explained by the assumption that the bacilli contained in the tuberculous tissue are mostly dead, or much more probably that an individual susceptibility to the disease is requisite for its productions.

The widespread idea, before the discovery of the leprosy bacillus, that the disease was associated with the constant eating of dried fish or a certain kind of food, has now been entirely abandoned.

The relation of leprosy to tuberculosis is sufficiently evident from their great similarity in many respects. This is rendered still more remarkable by the fact that leprosy reacts, both locally and generally, to an injection of tuberculin in the same manner as tuberculosis, but to a somewhat less extent.

Differential Diagnosis.—The differential diagnosis between leprosy and tuberculosis is not difficult in typical cases. The large numbers of bacilli found in the interior of the cells would point with great probability to leprosy. Too much importance should not be placed

upon the staining peculiarities, as these are not constant. Moreover, the two diseases not infrequently occur together in the same individual. In making the diagnosis, therefore, all the signs, histological and pathogenic, must be considered and animal inoculations made.

TIMOTHY AND OTHER GRASS BACILLI.

On various grasses, in cow's manure, in butter, and in milk there have been discovered a number of varieties of bacteria which have more or less of the characteristics of the tubercle bacillus. Some of them are as difficult to stain and as resistant to the decolorizing action of mineral acids and alcohol as the tubercle bacillus found in man. Many of them are of the same general size and shape as the tubercle bacillus, and, strangely enough, produce in animals small diseased areas which not only macroscopically but also microscopically resemble miliary tubercles due to the tubercle bacillus. They are, however, entirely different in their culture characteristics, producing in twenty-four to forty-eight hours, on ordinary culture media, moist, round colonies of an eighth to a quarter of an inch in diameter, and of a more or less intense pink color. In animals they produce only localized lesions, causing death only when injected in large numbers. The inoculated animals are unaffected by tuberculin injections. The chief interest which these bacilli have for us is the possibility of confusing them with tubercle bacilli. This danger is always present in milk, for grass bacilli find so many means of gaining entrance to it. In the examination of dust, healthy throat and nose secretions, etc., the simple microscopic examination might lead to error.

They can be separated from tubercle bacilli by inoculating animals in which no progressive lesions will develop. If there is any doubt about the nature of the infection, inject 2 c.c. of a standardized tuberculin, when if infected with tuberculosis they will die, but if by grass bacilli they will show little or no reaction. If a second group of guinea-pigs are inoculated with a small amount of the infected tissue there will develop progressive tuberculosis, if the doubtful bacilli were tubercle bacilli, and practically no lesions if they were grass bacilli. Cultures from the lesions will also show, on ordinary media, pink colonies if grass bacilli are present, and no growth if only tubercle bacilli.

CHAPTER XXV.

THE INFLUENZA AND PSEUDOINFLUENZA BACILLI—THE KOCH-WEEKS BACILLUS.

THE INFLUENZA BACILLUS.

INFLUENZA as a distinct entity can be traced back to the fifteenth century and probably existed at a much earlier date.

At times but few endemic cases occur, and then a great epidemic spreads over the civilized world. The last great epidemic reached Russia from the East in the fall of 1889 and gradually spread over Europe and to America, reaching the latter country in December of that year. Since then we have had more or less of it, especially during the winter months. Many acute inflammations of the respiratory mucous membranes, due to pneumococci and streptococci, give symptoms similar to those due to the influenza bacillus.

The rapidity of the spread of epidemics of influenza suggested that persons were the carriers of the infection, while the location of the disease pointed to the respiratory tract as the location of, and to the expectoration as the chief source of infection by, the microörganisms.

FIG. 113

Influenza bacilli. ×1100 diameters.

After numerous unsuccessful attempts, during the epidemic of 1889 and succeeding years, to discover the specific cause of influenza, Pfeiffer (1892) succeeded in isolating and growing upon blood agar a bacillus which abounded in the purulent bronchial secretion of patients suffering from epidemic influenza, which he showed was the probable cause of the disease.

Morphology.—Very small, moderately thick bacilli (0.2 to 0.3μ in thickness, to 0.5 to 2μ in length), usually occurring singly or united in pairs, but threads or chains of three, four, or more elements are occasionally found. No capsule has been demonstrated.

Staining.—The bacillus *stains* with difficulty with the ordinary aniline colors—best with diluted Ziehl's solution (water 9 parts to Ziehl's solution 1 part), or Loeffler's methylene-blue solution, with heat. When faintly stained the two ends of the bacilli are sometimes

more deeply stained than the middle portion. They are not stained by Gram's method.

Biology.—An aërobic, non-motile bacillus; does not form spores; no growth occurs with most cultures below 26° C., or above 41° C., or in the entire absence of oxygen.

Cultivation.—This bacillus is best cultivated at 37° C., and on the surface of ordinary nutrient culture media containing hæmoglobin. Plain or *glycerin agar*, or *blood serum* thinly streaked with rabbit, guinea-pig, or human blood, make the best media for its growth. At the end of eighteen hours in the incubator very small circular colonies are developed, which, under a low magnification (100 diameters), appear as shining, transparent, homogeneous masses, and even under a No. 7 lens scarcely show at all the individual organisms. Older colonies are sometimes colored yellowish-brown in the centre. A characteristic feature of the influenza bacillus is that the colonies tend to remain separate from each other, although when they are thickly sown in a film of moist blood upon nutrient agar they may become confluent. Transplantation of the original culture to ordinary agar or serum cannot, as a rule, be successfully performed, owing to the want of sufficient hæmoglobin; but if sterile rabbit, pigeon, or human blood be added to these media, transplantation may be indefinitely performed, provided it is done every three or four days. Cultures may remain alive up to seventeen days. By a series of beautifully carried out experiments, Pfeiffer showed that not only were the red blood cells the necessary part of the blood needed for the growth of the influenza bacillus, but that it was the hæmoglobin in the cells.

In *bouillon* in thin layers, to which blood is added, a good development takes place if there is free excess of oxygen.

Resistance and Length of Life.—The influenza bacillus is very sensitive to desiccation; a pure culture diluted with water and dried is destroyed with certainty in twenty-four hours; in dried sputum the vitality, according to the completeness of drying, is retained from twelve to forty-eight hours. It does not grow, and soon dies in water. In blood-bouillon cultures at 20° C. they retain their vitality for from a few days to two or three weeks. In moist sputum it is difficult to determine the duration of their life, since the other bacteria overgrow and make it impossible to find them. It is probable that they can remain alive for at least two weeks. The bacilli are very readily killed by chemicals, disinfectants, and succumb to boiling within one minute and to 60° C. within five minutes.

Detection of the Influenza Bacillus in Sputum.—The direct microscopic examination of stained smears of sputum may give considerable information as to the probable presence of influenza-like bacilli. In patients suffering from influenza the bacilli are found chiefly in the nasal and bronchial secretions. In acute uncomplicated cases they may be observed microscopically in large masses, and often in absolutely pure culture; the green, purulent sputum derived from the bronchial tubes is especially suitable for examination. The older the

process is, the fewer free bacilli will be found and the more frequently will they be seen lying within the pus cells, instead of being embedded free in the secretion as at first. At the same time they stain less readily and present more irregular and swollen forms. The frequent presence of other influenza-like bacilli in the throat secretions leads to so much doubt that it is advisable in important cases from the start to make use of plate cultures, the best medium being nutrient agar freshly smeared with a film of rabbit's blood.

Effect on Animals.—The bacillus of influenza, in so far as experiments show, produces a disease at all similar to influenza only in monkeys and to a less extent in rabbits. When a small quantity of culture on blood agar, twenty-four hours old, suspended in 1 c.c. of bouillon, was injected intravenously into rabbits, Pfeiffer found a characteristic pathogenic effect was produced. The first symptoms were developed in one and a half to two hours after the injection. The animals became extremely feeble, lying flat upon the floor, with their limbs extended, and suffered from extreme dyspnœa. The temperature rose to 41° C. or above. At the end of five or six hours they were able to sit up on their haunches again, and in twenty-four hours had recovered. Larger doses caused the death of the animals inoculated. These results are attributed by Pfeiffer to toxic products present in the cultures, and in none of his experiments was he ever able to obtain effects resembling septicæmic infection. Cultures killed by moderate heat give much the same results. In some of the experiments on monkeys, these animals, when cultures were rubbed into the nasal mucous membrane, showed a febrile condition, lasting for a few days; but in no instance has Pfeiffer observed a multiplication of the bacilli introduced.

The cell bodies of the bacilli seem to possess considerable pyogenic action.

Immunity.—Possibly an immunity for a short period against the influenza poison may be established after an attack. At least in three experiments made by Pfeiffer on monkeys, these animals, after recovering from an inoculation with bacilli, seemed to be much less susceptible to a second injection.

Pathogenesis for Man.—The invasion of the body by the influenza bacillus is chiefly confined to the respiratory passages. Very frequently the influenza process invades portions of the lung tissue. In severe cases a form of pneumonia is the result, which is lobular and purulent in character and accompanied by symptoms which may be somewhat characteristic for influenza, or, again, almost identical with bronchopneumonia due to the pneumococcus. The walls of the bronchioles and alveolar septa become densely infiltrated with leukocytes, and the spaces of the bronchial tubes and alveoli become filled. The influenza bacilli are found crowded in between the epithelial and pus cells and also penetrate the latter. There may be partial softening of the tissues or even abscess formation. Bacilli are found in fatal cases to have penetrated from the bronchial tubes not only

into the peribronchitic tissue, but even to the surface of the pleura, and rarely they have been obtained in pure cultures in the pleuritic exudation. The pleurisy which follows influenza, however, is usually a secondary infection, due to the streptococcus or pneumococcus.

Presence in Blood.—Bacilli that resemble influenza bacilli so closely as to make their separation difficult or almost impossible are found at times in the blood during the early days of an acute infection; and sometimes in bad cases in young children a septicæmia develops before death. Whether the typical influenza bacillus is found in the blood as supposed by Canon is still a matter of controversy. It is found at times in otitis media accompanying influenza, and has been found in the meninges in cases of meningitis. So far as positive results have shown, influenza would seem to be almost always a local infection confined chiefly to the air passages. The general, cerebral, gastric, and other symptoms produced are due to the absorption of the toxic products of the specific organism, these poisons being particularly active in their effects on the central nervous system.

Presence of Influenza Bacilli in Chronic Influenza and in Tuberculosis.—Ordinarily influenza runs an acute or subacute course, and not infrequently it is accompanied by mixed infections with the pneumococcus and streptococcus. Pfeiffer was the first to draw attention to certain chronic conditions depending upon the influenza bacillus. Bacilli may be retained in the lung tissue for months at a time, remaining latent a while, and then becoming active again, with a resulting exacerbation of the disease. Consumptives are liable to carry influenza bacilli for years and are particularly susceptible to attacks of influenza. Williams, in the examination of sputa in cases of pulmonary tuberculosis, has found abundant influenza bacilli to be present in a large proportion of the samples of sputum from consumptives, and this not only in winter but also in the summer, when no influenza was known to be present in New York. Taken together with results elsewhere, this indicates that at all times of the year many consumptives carry about with them influenza bacilli, and that very likely many healthy persons as well as persons suffering from bronchitis also harbor a few. Given proper climatic conditions, we have at all times the seed to start an epidemic.

Epidemiology.—The discovery of this bacillus enables us to explain many things, previously unaccountable, in the cause of epidemic influenza. We now know, from the inability of the influenza bacillus to exist for long periods in dust, that the disease is not transmissible to great distances through the air. We also know that the infective material is contained only in the catarrhal secretions. Sporadic cases or the sudden eruption of epidemics in any localities from which the disease has been absent for a long time, or where there has been no new importation of infection, may possibly be explained by assuming that the bacilli, as already mentioned, often remain latent in the lungs or bronchial secretions of the body for many months, and per-

haps years, and then become active again, when under favorable circumstances they may be communicated to others.

Bacteriological Diagnosis.—This is of importance for the identification of clinically doubtful cases, which, from their symptoms, may be mistaken for bronchitis, pneumonia, or tuberculosis. Up to the present time the diagnosis gives us little help in treatment.

In acute uncomplicated cases the probable diagnosis can be frequently made by microscopic examinations of stained preparations of the sputum. In chronic cases or those of mixed infection few or many bacilli may be found and the culture method may be necessary to give even a probable diagnosis. The bacillus of influenza is not readily separated by its morphological, staining, and cultural peculiarities from other bacteria belonging to the influenza group, and at present it is almost impossible to identify it certainly.

The Pseudoinfluenza Bacillus.—This bacillus is culturally very similar to the typical influenza bacillus, but may be distinguished from it by its larger size and tendency to grow out into long threads. It is not certain but that it is a form of the influenza bacillus.

Other Bacilli Resembling the Influenza Bacillus.—There are a number of bacilli which differ slightly in morphology and growth in culture from the characteristics of the typical influenza bacillus. One of these strains is regulary found in whooping-cough. It produces, when injected in animals, agglutinins which are specific for them, but not for influenza bacilli. Both bacilli have in common group agglutinins (Wollstein). The blood of those suffering from whooping-cough usually agglutinates the whooping-cough bacilli, but not the influenza bacilli. Further investigation is required to establish their significance in the disease (see, too, p. 484 for the bacillus of Bordet and Gengou). The Koch-Weeks bacillus is also very similar to the influenza bacillus.

Relation of the Clinical Symptoms to the Bacterial Excitant.—There is no doubt that other infections are also included under the clinical forms of influenza, and during an epidemic of bronchopneumonias, irregular types of lobar pneumonias, and cases of bronchitis frequently have symptoms so closely alike that the nature of the bacteria active in the case is very frequently different from that supposed by the clinician. Thus in four consecutive autopsies examined by the writer the influenza bacillus was found almost in pure culture in one case believed, from the symptoms, to be due to the pneumococcus, and entirely absent in two of the three believed to be due to it. Except for these examinations the clinician would be of the opinion that he had clearly diagnosed bacteriologically the cases, while in fact he had been wrong in three of the four.

The striking symptoms in acute respiratory diseases are frequently more due to the location of the lesions than to the special variety of organisms producing them. In epidemics of influenza there are, of course, many cases which, on account of their characteristic symptoms, can be fairly certainly attributed to the influenza bacilli. Even

under these circumstances error may be made, as, for instance, two cases of apparently typical influenza were reported in a household and both showed a total absence of influenza bacilli. The pneumococcus was present in almost pure culture.

Examination of Sputum for Influenza Bacilli.—1. Sputum coughed from the deeper air passages and not from throat scraping should be used.

2. The sputum should be received in a sterile bottle, which should then be placed immediately in cracked ice.

3. Blood-agar plates should be made by dropping a drop of fresh rabbit's blood, obtained aseptically, on the centre of a hardened agar plate.

4. One of the more solid masses of the sputum should be taken from the bottle with sterile forceps and placed on a plain agar plate. A small portion of this mass should be separated with a sterile platinum needle and drawn through the blood on the blood-agar plate from the centre out in different directions. The larger part of what is left of this small portion is then placed in a similar manner over a second blood agar, and from this to a third, sterilizing the needle between the transfers. The plate should be placed in the thermostat for twenty-four hours.

5. After the plates are planted two smears should be made, one stained by Gram and the other by weak carbol-fuchsin.

6. After twenty-four hours the plates are examined under low power. The influenza colonies use up the hæmoglobin, and in parts of the blood-agar plate where the blood is of right thickness such colonies show as almost clear areas surrounded by the red blood. With a higher power (No. 6 or 7 objective), if such areas seem to be made up of fine indefinite granulations, they are practically sure to be influenza colonies. Most influenza colonies are more highly refractive than other light colonies, and they show this characteristic best when they grow on the edge of a blood mass. Many influenza colonies also show heapings in the centre. Influenza colonies growing away from the blood cells are less characteristic in appearance and less easily differentiated from other similar bacteria.

7. Fishings from the influenza-like colonies should be planted on blood-agar tubes, and if, after twenty-four hours in the thermostat, the resulting growth should consist of influenza-like organisms, plantings should be made on plain agar. The first generation on plain agar may show slight growth because of the blood carried over from the original tube, but the second generation should show no growth if the organism is the influenza bacillus.

8. The agglutination characteristics of the cultures should be tested in the serum from a rabbit injected with a single typical culture, and in the serum from one injected with a number of cultures. The agglutination tests should be carried out in order to gain information. The cultures tested in the Research Laboratory have shown considerable variation.

For Testing the Agglutination of Influenza Bacilli in the Hanging Drop.—Grow the cultures on slanted agar tubes to which, after cooling to 40° C., ½ c.c. of defibrinated blood has been added. When twenty to twenty-four hours old, make a suspension of the bacilli in normal salt solution, controlling the number of bacilli by examining a hanging-drop preparation. The influenza bacilli seem to agglutinate rather slowly, so it usually takes four to five hours to get a good reaction.

Serum Therapeutics.—No protective serum has been produced which has any value in treatment.

Vaccines.—These have not been proven to be of value.

THE KOCH-WEEKS BACILLUS OF CONJUNCTIVITIS.

This bacillus was first observed by R. Koch in 1883 while making certain investigations into inflammation of the eye occurring during an epidemiç of cholera in Alexandria. It was later, in 1887, more specifically described by Weeks[1] in New York. Weeks obtained it in pure culture in 1890.

The infective disease which is caused by this bacillus seems to be very widely distributed, no land or clime probably being exempt from it. In this country it occurs epidemically and with increasing frequency during the spring and fall months. Weeks has found the bacillus in over 1000 cases. This disease is known as pink eye.

Morphology.—The bacilli from the purulent secretions are small and slender, being not unlike the influenza bacilli but somewhat longer. The shorter bacilli not infrequently have the appearance of

FIG. 114

Koch-Weeks Bacillus (pink-eye)—3d generation. × 1000 diameters. (Weeks.)

FIG 115

Secretion of muco-pus from conjunctiva in "pink-eye." × 1000 diameters. (Weeks.)

diplococci. Sometimes they exhibit slight polar staining. Their width is very constant. The ends are rounded. They are rapidly decolorized by Gram.

Staining.—They are best stained by very dilute solutions of carbol-fuchsin or Loeffler's methylene blue, but do not stain readily.

In smear preparations the Koch-Weeks bacilli are, as a rule, seen alone or associated with isolated cocci and bacilli, especially xerosis bacilli. They are not infrequently observed within the cells, and are very rarely associated with gonococci and pneumococci, such mixed infections being extremely uncommon.

Biological Characters.—The Koch-Weeks bacillus grows only at temperatures near to 37° C. Of the ordinary culture media none but moist and slightly alkaline peptone agar can be employed. The best results have been obtained with serum agar or a mixture of glycerin agar and ascitic fluid, 2 to 1. Pure cultures are rarely obtained at first;

[1] Weeks. N. Y. Med. Rec., 1887, xxxi., page 571.

they are usually associated with colonies of xerosis bacilli or staphylococci. After twenty-four to forty-eight hours the colonies are noticeable as moist, transparent, shining drops or points. Microscopically examined under low magnifying power they appear like small gas bubbles; by closer examination they are seen to be round, lying loosely on the surface, and are readily removed. Under higher magnification a number of fine points are observable. The colonies, which resemble those of influenza, have a tendency to confluesce, but are not so sharply defined as the latter and become more quickly indistinguishable. Isolated colonies, especially those in the neighborhood or xerosis bacilli or staphylococci, grow larger and their contour is slightly wavy; they are more opaque and granular than influenza colonies. In serum or blood bouillon a slight cloudiness is produced which finally settles down.

Resistance.—In culture media the bacilli die rapidly, seldom living more than five days. They resist a temperature of 50° for ten minutes, but cannot withstand 60° for more than one or two minutes. They cannot resist drying for any length of time.

Transmission.—This occurs only by contact either by direct or indirect conveyance of the moist infective material. Infection is not communicated through the air by means of dust, as the bacilli soon die when dried. It may, however, be conveyed by flies, etc.

Pathogenesis.—The Koch-Weeks bacillus is not pathogenic for animals. Man, on the contrary, is extremely susceptible to infection from this bacillus, which produces one of the most contagious diseases known.

Immunity.—Immunity is not produced to any extent by one attack, but there does seem to be an individual susceptibility to the disease.

Differential Diagnosis.—The only microörganisms from which the Koch-Weeks bacillus would seem to require differentiation are those of the influenza group. These latter bacilli, however, grow well only on hæmoglobin media, which the Koch-Weeks bacillus does not require. The colonies on serum agar are smaller than those of the influenza bacilli and the edges more granular. While the influenza bacillus is slightly pathogenic for certain animals, the Koch-Weeks bacillus has so far given negative results with all animals.

CHAPTER XXVI.

THE PYOGENIC COCCI.

THE STAPHYLOCOCCI.

STAPHYLOCOCCI were first obtained from pus by Pasteur in 1880. In 1881 Ogston showed that they frequently occurred in abscesses, and in 1884 Rosenbach fully demonstrated their etiological importance in circumscribed abscesses, osteomyelitis, etc. Of the staphylococci those producing yellow and white pigments are by far the most important since they are the pathogenic varieties.

The Staphylococcus Pyogenes Aureus.—The *Staphylococcus aureus* is one of the commonest pathogenic bacteria, being usually present in the skin and mucous membranes, and is the organism most frequently concerned in the production of acute, circumscribed, suppurative inflammations.

Morphology.—Small, spherical cells, having a diameter of 0.7μ to 0.9μ, occurring solitary, in pairs as diplococci, in short rows of three or four elements, or in groups of four, but most commonly in irregular masses, simulating clusters of grapes; hence the name *staphylococcus*. (See Fig. 116.)

Staining.—It *stains* quickly in aqueous solutions of the basic aniline colors and with many other dyes. When previously stained with aniline gentian violet it is not decolorized by Gram's method. When slightly stained each sphere frequently is seen to be already dividing into two semispherical bodies.

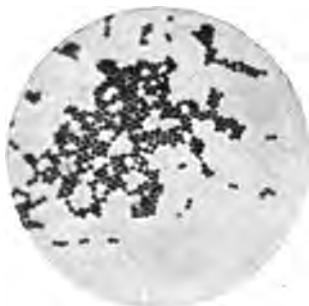
Biology.—The *Staphylococcus pyogenes aureus* is an aërobic, *facultative anaërobic* micrococcus, growing at a temperature from 8° to 43° C., but best at 25° to 35° C. The staphylococci grow readily on all the common laboratory media, such as milk, bouillon, nutrient gelatin, or agar. A slightly alkaline reaction to litmus is best for the growth of the staphylococci, but they also grow in slightly acid media.

Cultivation.—Growth in Nutrient Bouillon.—The growth of the staphylococcus is rapid, reaching about 50,000,000 per c.c. at the end of twenty-four hours at 30° C. The bouillon is cloudy and frequently has a thin pellicle. Later a slimy sediment forms. The odor is disagreeable. In peptone-water, growth occurs with indol production.

Growth on Gelatin.—Grown on gelatin plates it develops, at room-temperature, within forty-eight hours, punctiform colonies, which when examined under a low-power lens, appear as circular disks of a pale-brown color, somewhat darker in the centre, and surrounded by a smooth border. The colonies grow rapidly. The appearance of the

growth is most characteristic. Immediately surrounding the colonies, which are of a pale yellow color, there is a deepening of the surface of the gelatin, due to its liquefaction. By suitable light a number of these shallow depressions with sharply defined outlines may be seen on the gelatin plate, having a diameter of from 5 to 10 mm., in the centres of which lie the yellow colonies. Later the liquefaction becomes general, the colonies running together. In stab cultures in gelatin a white confluent growth at first appears along the line of puncture, followed by liquefaction of the medium, which rapidly extends to the sides of the test-tube. At the end of two days the yellow pigmentation begins to form, and this increases in intensity for eight days. Finally, the gelatin is completely liquefied, and the staphylococci form a golden-yellow or orange-colored deposit at the bottom of the tube. Under unfavorable conditions the staphylococcus aureus gradually loses it ability to make pigment and to liquefy gelatin.

FIG. 116



Staphylococcus. × 1100 diameters.

Growth on Agar.—In streak and stab cultures on agar a whitish growth is at first produced, and this at the end of a few days becomes a faint to a rich golden-yellow on the surface. The yellow pigmentation is produced only in the presence of oxygen; colonies found at the bottom of a stab culture or under a layer of oil remain white.

Milk.—Milk is coagulated at the end of from one to eight days.

Potato.—The staphylococci grow readily on potato and produce abundant pigment.

Growth on Loeffler's Solidified Blood Serum.—Growth vigorous, with fairly good pigment production. Some varieties slowly liquefy the serum.

Growth on Blood Agar.—If nutrient agar to which a little animal blood has been added is streaked with staphylococci there appears, at the end of twenty-four hours at 35° C., about the growth a clear zone, owing to the hæmolytic effect of the staphylococcus products.

Acids Produced.—In certain culture media, as a result of the growth of the staphylococcus aureus, there is a *production of acid* in considerable quantities, these consisting chiefly of lactic, butyric, and valerianic acids. These acids have been supposed to play a part in the production of pus, in which, according to some observers, they are often present.

Resistance.—The staphylococcus is distinguished from most other non-spore-bearing pathogenic bacteria by its greater power of resistance to outside influences, desiccation, etc., as well as to chemical disinfectants. Cultures of the staphylococcus pyogenes in gelatin or agar retain their vitality for a year or more. Suspended in water its thermal death point varies with different cultures and averages about two hours at 50° C., one-half hour at 60° C., ten minutes at 70° C.,

and five minutes at 80° C. Upon silk threads and in media rich in organic matter its resistance is greater, but subjected to 80° C. for thirty minutes or boiling for two minutes it is almost surely killed. Cold has but little effect. Thirty per cent. of the organisms remained alive after being subjected by us to freezing in liquid air for thirty minutes. These are average figures. Some cultures are more resistant than others.

They are quite resistant to direct sunlight and drying. Dried pus contains living staphylococci for weeks and even months, and they can be found alive in the fine dust of the air in living and in operating-rooms.

To most disinfectants the staphylococci are rather resistant. The presence with staphylococci of organic substances, especially albumin, increases their resistance. In watery solution dissolved mercuric chloride, 1:1000, destroys the organisms in five to fifteen minutes, but when in pus not for several hours. Hydrogen peroxide in 1 per cent. solution kills in about one-half hour.

Products of Growth.—Besides the lipochrome and gelatin liquefying enzyme, there are produced other enzymes. The specific hæmolysin, known as staphylolysin is destroyed by heating for twenty minutes at 56° C. An antibody for this is formed by inoculating animals with culture filtrates. A substance called leukocidin is produced which injures leukocytes. It also produces an antibody.

Toxic Substances.—Filtrates of cultures contain toxic substances. Injected into the peritoneal cavity they excite peritonitis. Under the skin they produce infiltration or abscess formation. In the blood they injure both the red and white corpuscles.

Cultures of the staphylococcus, when sterilized by boiling and injected subcutaneously, produce marked positive chemotaxis and often local abscesses. Leber found also that sterilized cultures introduced into the anterior chamber of the rabbit's eye would bring about a fibro-purulent inflammation, the cornea becoming infiltrated, and perforation alongside of the sclerotic ring finally taking place. This was followed by the formation of pus in the anterior chamber and recovery. These local changes follow the inoculation of small quantities only of the dead cultures; but when large amounts are injected into a vein or into the abdominal cavity, toxic effects are produced. The hæmolytic effects of certain products of virulent staphylococci have recently been studied. In cultures they can be detected about the third or fourth day of incubation and reach their maximum on the ninth to fourteenth day. Virulent staphylococci are more apt to produce this substance than the non-virulent, but there is no definite rule.

Pathogenesis.—The pathogenic effect of the *Staphylococcus pyogenes aureus* on test animals varies considerably, according to the mode of application and the virulence of the special culture employed. In man a simple rubbing of the surface of the unbroken skin with pus from an acute abscess is, as a rule, sufficient to produce a purulent

inflammation, and the introduction of a few germs from a septic case into a wound may lead to a fatal pyæmia. These conditions can only be reproduced in lower animals with difficulty, and by the inoculation of large quantities of the culture. Small subcutaneous injections, or the inoculation of open wounds in mice, guinea-pigs, and rabbits, are commonly without result; occasionally abscess formation may follow at the point of inoculation, which usually ends in recovery. The pus-producing property of the organism is exhibited in proportion to the virulence of the culture employed. Slightly virulent cultures, which constitute the majority of those obtained from pus taken from the human subject, when injected subcutaneously in large quantities (several c.c. of a fresh bouillon culture) in rabbits or guinea-pigs, give rise to local pathological lesions—acute abscesses. When virulent cultures are used—which are rarely obtainable—0.5 c.c. of a fresh bouillon culture is sufficient to produce similar results. The abscesses generally heal without treatment; sometimes the animals die from marasmus in consequence of the suppurative process. In intraperitoneal inoculations the degree of virulence of the culture employed is still more conspicuous in the effects produced. The animals usually die in from two to nine days. The most characteristic pathological lesions are found in the kidneys, which contain numerous small collections of pus, and under the microscope present the appearances resulting from embolic nephritis. Punctiform, whitish-yellow masses of the size of a pea are found permeating the pyramids. Many of the capillaries and some of the smaller arteries of the cortex are plugged up with thrombi, consisting of micrococci. Metastatic abscesses may also be observed in the joints and muscles. The micrococci may be recovered in pure cultures from the blood and the various organs; but they are not numerous in the blood and are often difficult to demonstrate microscopically. Intravenous inoculations of animals are followed by similar pathological changes. Orth and Wyssokowitsch first pointed out that injection of staphylococci into the circulation of rabbits whose cardiac valves have previously been injured produced ulcerative endocarditis. Subsequently, Weichselbaum, Prudden, and Fraenkel and Sänger obtained confirmatory results, thus establishing the fact that when the valves are first injured, mechanically or chemically, the injection into a vein of a pure culture of staphylococcus aureus gives rise to a genuine ulcerative endocarditis. It has been further shown by Ribbert that the same result may be obtained without previous injury to the valves by injecting into a vein the staphylococcus from a potato culture suspended in water. In his experiments not only the micrococci from the surface, but the superficial layer of the potato were scraped off with a sterilized knife and mixed with distilled water and the successful result is ascribed to the fact that the little agglomerations of micrococci and infected fragments of potato attach themselves to the margins of the valves more readily than isolated cocci would do. Not infrequently, also, in intravenous inoculations of young animals there occurs a localization of the injected material in the

marrow of the small bones. This may take place in full-grown animals when the bones have been injured or fractured. The experimental osteomyelitis thus produced has been demonstrated to be anatomically analogous to this disease in man.

Occurrence in Man.—Practically all microörganisms have been shown by experiment to produce, under certain conditions, the formation of pus by their products when inoculated into the animal body; but, while this has been demonstrated, the researches of bacteriologists show that only a few species are usually concerned in the production of acute abscesses in man. Of these the two most important, by reason of their frequent occurrence and pathogenic power, are *Staphylococcus pyogenes* and *Streptococcus pyogenes*. These two organisms are often found in the same abscess; thus, Passet, in 33 cases of acute abscess, found *Staphylococcus aureus* and *albus* associated in 11, *albus* alone in 4, *albus* and *citreus* in 2, *Streptococcus pyogenes* alone in 8, *albus* and *Streptococcus* in 1, and *albus*, *citreus*, and streptococcus in 1. The staphylococcus is liable to enter as a mixed infection into most infections due to other bacteria, and is almost always met with in all inflammations of the skin and mucous membranes or in cavities connected with them.

The staphylococcus (staphylococcus aureus) has been demonstrated not only in furuncles and carbuncles, but also in various pustular affections of the skin and mucous membranes—impetigo, sycosis, purulent conjunctivitis and inflammation of the lacrymal sac; in acute abscesses formed in the lymphatic glands, the parotid gland, the tonsils, the mammæ, etc.; in metastatic abscesses and purulent collections in the joints; in empyema, infectious osteomyelitis, ulcerative endocarditis, pyelonephritis, abscess of the liver, phlebitis, etc. It is one of the chief etiological factors in the production of pyæmia in the various pathological forms of that condition of disease. It is remarkable how many staphylococci may be present in the blood without a fatal result, if the original source of infection is removed. We met with one case in which over 800 staphylococci were present in 1 c.c. of blood. A week later only five were found. The patient finally died from pneumonia.

Not all persons are equally susceptible to infection by the staphylococcus; those who are in a cachectic condition or suffering from constitutional diseases, like diabetes, are especially predisposed to infection. In healthy individuals certain parts of the body, as the back of the neck and the buttocks, are more liable to be attacked than others, with the production of furuncles, carbuncles, etc. In persons in whom sores are readily caused, in consequence of disturbances of nutrition, as in exhausting diseases, the micrococci settle at the points of least resistance. Such conditions are present in the bones of debilitated young children, in fractures, and in injuries in general.

The pyogenic properties of the staphylococcus have been demonstrated upon man by numerous experiments. Garré inoculated a small wound at the edge of one of his finger-nails with a minute quantity of a pure culture, and purulent inflammation, extending around

the margin of the nail, resulted from the inoculation. *Staphylococcus aureus* was recovered in cultures from the pus thus formed. The same observer applied a considerable quantity of a pure culture obtained from this pus—third generation—to the unbroken skin of his forearm, rubbing it well into the skin. At the end of four days a large carbuncle, surrounded by isolated furuncles, developed at the point where the culture had been applied. This ran the usual course, and it was several weeks in healing. No less than seventeen scars remained to testify to the success of the experiment.

Immunity.—Rabbits have been rendered immune by means of inoculations with both dead and living cultures. Unless the inoculations are carefully done the animals frequently succumb. The staphylococci injected into an immunized animal are more rapidly taken up by the leukocytes than when injected into an untreated animal.

A serum having some protective power has also been elaborated.

Therapeutic Use of Vaccine.—The treatment of abscesses, boils, and other localized staphylococcus infections by injections of repeated doses of one hundred to three hundred million staphylococci has given very successful results. Pyæmias have also been treated, but with uncertain results. The serum has not been used with success.

Staphylococcus Pyogenes Albus.—It is morphologically identical with the *Staphylococcus pyogenes aureus*, and is probably the same organism which has lost the property of producing pigment. On the average it is somewhat less pathogenic and seldom produces pyæmia or grave infections. The surface cultures upon nutrient agar and potato have a milk-white color. Its biological characters are not to be distinguished from the *Staphylococcus aureus*.

The majority of bacteriologists agree with Rosenbach, that the aureus is found at least twice as frequently in human pathological processes as the albus.

Staphylococcus Epidermidis Albus (Welch.)—Probably identical with the *Staphylococcus pyogenes albus*. With reference to this micrococcus, Welch says: "So far as our observations extend—and already they amount to a large number—this coccus may be regarded as nearly, if not quite, a constant inhabitant of the epidermis. It is now clear why I have proposed to call it the *Staphylococcus epidermidis albus*. It possesses such feeble pyogenic capacity, as is shown by its behavior in wounds as well as by experiments on rabbits, that the designation *Staphylococcus pyogenes albus* does not seem appropriate. Still, I am not inclined to insist too much upon this point, as very probably this coccus—which has hitherto been unquestionably identified by others with the ordinary *Staphylococcus pyogenes albus* of Rosenbach—is an attenuated or modified form of the latter organism, although, as already mentioned, it presents some points of difference from the classical description of the white pyogenic coccus."

According to Welch, this coccus differs from the *Staphylococcus albus* in the fact that it liquefies gelatin more slowly, does not so quickly cause coagulation in milk, and is far less virulent when in-

jected into the circulation of rabbits. It has been shown by the experiments of Bossowski and of Welch that this microörganism is very frequently present in aseptic wounds, and that usually it does not materially interfere with the healing of wounds, although sometimes it appears to cause suppuration along the drainage-tube, and it is the common cause of "stitch abscess."

Staphylococcus Pyogenes Citreus and other Staphylococci.—Isolated by Passet (1885) from the pus of acute abscesses, in which it is occasionally found in association with other pyogenic cocci. It is distinguished from the other species only by the formation of a lemon-yellow pigment.

Many other varieties of staphylococci have been occasionally met with which differ in some respects from the typical varieties. This difference may be in the fact that they liquefy gelatin more slowly or not at all, or in pigment formation, or in agglutination, or in still other respects. None of these varieties are of great importance.

FIG. 117

Micrococcus tetragenus. ×1000 diameters.

The Micrococcus Tetragenus.—This organism was discovered by Gaffky (1881). It is not infrequently present in the saliva of healthy individuals and in the sputum of consumptive patients. In sputum it is sometimes an evidence of mouth contamination rather than lung infection. It has repeatedly been observed in the walls of cavities in pulmonary tuberculosis associated with other pathogenic bacteria, which, though playing no part in the etiology of the original disease, contribute, doubtless, to the progressive destruction of the lung. Its pyogenic character is shown by its occasional occurrence in the pus of acute abscesses. Its presence has also been noted in the pus of empyema following pneumonia.

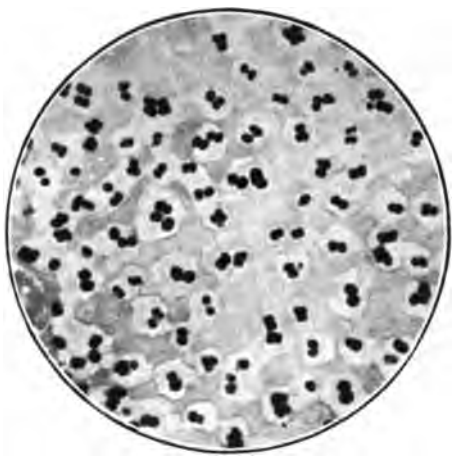
Morphology.—Micrococci having a diameter of about 1μ, which divide in two planes, forming tetrads, and bound together by a transparent, gelatinous substance, enclosing the cell like a capsule. In cultures the cocci are seen in various stages of division as large, round, cells, in pairs of oval elements, and in groups of three and four (Figs. 117 and 118). When the division is complete they remind one of sarcinæ in appearance, except that they do not divide in three directions and are not built up like diminutive cotton bales.

Staining.—This micrococcus *stains* readily with the ordinary aniline dyes; the transparent gelatinous envelope is only feebly stained. It is not decolorized by Gram's method.

Biology.—The growth of this micrococcus is slow under all conditions. It grows both in the presence and absence of oxygen; it grows best from 35° to 38° C., but may be cultivated also at the ordinary room-temperature—about 20° C.

Growth on Gelatin.—On gelatin plates small, white colonies are developed in from twenty-four to forty-eight hours, which, when examined under a low-power lens, are seen to be spherical or lemon-shaped, grayish-yellow disks, with a finely granular or mulberry-like surface, and a uniform but somewhat roughly dentated border. When the deep colonies push forward to the surface of the gelatin they form white, elevated, drop-like masses, having a diameter of 1 to 2 mm. In gelatin stick cultures the gelatin is not liquefied.

FIG. 118



Micrococcus tetragenus from peritoneal fluid. Stained with fuchsin. (Fraenkel.) ×1000 diameters.

Growth on Agar and Blood Serum.—The colonies appear as small transparent, round points, which have a grayish-yellow color and are slightly elevated above the surface of the medium.

Pathogenesis.—Subcutaneous injections of a culture of this micrococcus in minute quantity is usually fatal to white mice. The micrococci are found in comparatively small numbers in the blood of the vessels and heart, but are more numerous in the spleen, lungs, liver, and kidneys. Intraperitoneal injections given to guinea-pigs and mice are followed by purulent peritonitis, beautifully formed cocci in groups of four being obtained in immense numbers from the exudate. Rabbits and dogs are not affected by large doses of a culture subcutaneously or intravenously administered.

The serum from immunized cases has not been used therapeutically in human infection. Vaccines may be employed as with staphylococci.

THE STREPTOCOCCI.

Under this name must be included not only the streptococci which excite inflammation in man, but all spherical bacteria which divide, as a rule, in one plane only and remain attached in longer or shorter chains. This name comprises by no means so many varieties of bacteria as are grouped under the title bacilli. There are, nevertheless, a considerable number of distinct groups of streptococci which differ decidedly both in their cultural characteristics and their pathogenic properties. The streptococci average about 1μ in diameter. None of them forms spores or is motile. They are rather easily killed by disinfectants. Those that are pathogenic rarely reproduce themselves outside the bodies of man and animals.

Streptococcus Pyogenes.—The group of streptococci which in its importance as related to human infections outweighs all other streptococci is that which comprises the streptococci which excite erysipelas, many cases of cellulitis, abscess, septicæmia, pneumonia, etc., and passes under the name of *Streptococcus pyogenes.*

This organism was first discovered by Koch in stained sections of tissue, attacked by septic processes, and by Ogston in the pus of acute abscesses (1882). It was obtained by Fehleisen (1883) in pure cultures from a case of erysipelas, its cultural and pathological characters studied and demonstrated by him to be capable of producing erysipelas in man. Rosenbach (1884) and Krause and Passet (1885) isolated the streptococcus from the pus of acute abscesses and gave it the name of *Streptococcus pyogenes.* It has since been proved to be one of the chief etiological factors in the production of many suppurative inflammations. Formerly the streptococci of erysipelas, acute abscesses, septicæmia, puerperal fever, etc., were thought to belong to different species, because they were observed to possess apparent differences in their biological and pathological characteristics, according to the source from which they were obtained. Thus one species of streptococcus was believed to be capable of causing erysipelas only, another only acute abscesses; another sepsis, etc., but it is now known that the slight differences between the majority of these streptococci are but acquired non-permanent variations of organisms derived from the same species.

Morphology.—The cocci, when fully developed are spherical or oval. They have no flagella or spores. They vary from 0.4μ to 1μ

FIG. 119

Streptococci in peritoneal fluid, partly enclosed in leukocytes. ×1000 diameters.

FIG. 120

Streptococcus growing in long chains in bouillon culture. ×1000 diameters.

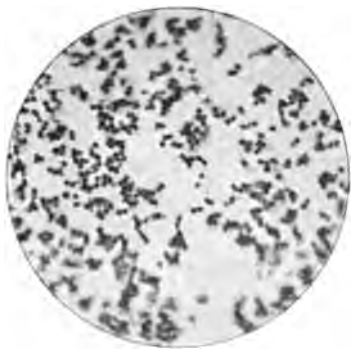
in diameter. They vary in dimensions in different cultures and even in different parts of a single colony. They multiply by binary division in one direction only, forming chains of eight, ten, twenty, and more elements, being, however, often associated distinctly in pairs. On solid media the cocci occur frequently as diplococci, but usually they grow in longer or shorter chains. Certain cocci frequently exceed their fellows greatly in size, especially in old cul-

tures, when this may be considered to be the result of involution forms. These were formerly called by Hueppe arthrospores. Some varieties have distinct capsules when growing in the blood and in blood-serum media (Hiss).

Staining.—They *stain* readily by aniline colors and the pyogenic varieties positively by Gram's method. Some varieties, mostly saprophytic, growing in short chains are negative to Gram's stain.

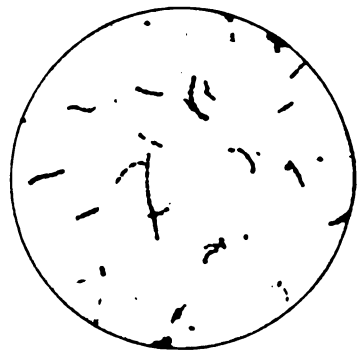
Biology.—Streptococci grow readily in various liquid and solid culture media. The most favorable temperature for their development is from 30° to 37° C., but they multiply rather freely at ordi-

FIG. 121



Streptococci from solidified serum culture appearing mostly in diplococci. ×1000 diameters.

FIG. 122



Streptococci in throat exudate smeared on cover-glass. ×1000 diameters

nary room temperature—18° to 20° C. They are facultative anaërobes, growing both in the presence and absence of oxygen.

Cultivation.—Growth on Gelatin.—Tubes of gelatin which have been inoculated with streptococci by puncture with platinum needle show on the surface no growth beyond the point of entrance. In the depth of the gelatin on the second or third day a distinct, tiny band appears, with granular edges or made up of granules. These granules may be very fine or fairly coarse. They are nearly translucent, with a whitish, yellowish, or brownish tinge. With characteristic cultures the gelatin is *not liquefied.*

Growth on Agar.—On agar plates the colonies are visible after twelve to thirty hours' growth at 37° C., and present a beautiful appearance when magnified sufficiently to see the individual cocci in the chain. The colonies are small, not averaging over 0.5 mm. in diameter (pin head). From different sources they vary in size, thickness, mottling, color, and in the appearance of their borders. The streptococcus growing in short chains in bouillon shows but little tendency to form true loops, but rather projecting rows at the edges of the colonies, while those growing in long chains show beautiful loops, which are characteristic of this organism.

Growth in Bouillon.—Most streptococci grow well in slightly alka-

line bouillon at 37° C., reaching their full development within thirty-six to forty-eight hours. Those which grow in long chains usually give an abundant flocculent deposit and leave their liquid clear. The deposit may be in grains, in tiny flocculi, in larger flakes, or in tough, almost membranous masses, the differences depending on the strength of union between the pairs of cocci in the chains. Some of the streptococci growing in long chains, however, cause the broth to become cloudy. This cloudiness may be only temporary or it may be lasting. Those growing in short chains, as a rule, cloud the broth, this cloudiness remaining for days or weeks. A granular deposit appears at the bottom of the tube. An addition of 0.5 to 1 per cent. glucose aids the development of streptococci, but the acid produced tends later to hasten their death and make them lose virulence. A trace of calcium aids the growth. This is best added as a piece of marble, which has the additional advantage of neutralizing some of the acids produced.

Growth in Ascitic or Serum Bouillon.—The development in this, which is the best medium for the growth of the streptococcus, is more abundant than in plain bouillon. The liquid is generally clouded, and a precipitate occurs after some days, the fluid gradually clearing. The addition of blood serum frequently causes streptococci, growing in short chains in nutrient bouillon, to produce long chains. The reverse is also true, and in the blood all forms are usually found, partly, at least, as diplococci or in short chains.

Effect on Inluin.—This is not fermented by most varieties.

Growth on Solidified Blood Serum.—This is also an excellent medium for the streptococcus. Tiny, grayish colonies appear twelve to eighteen hours after inoculation.

Growth in Milk.—All streptococci grow well in milk. As a rule, when growth is luxuriant a marked production of lactic acid with coagulation of the casein occurs.

Development of Hæmolytic Substances.—Most streptococci produce these. This is especially true of those from human septic infections. As the pneumococci and some types of streptococci produce them in a much less degree, blood-agar plates are a very useful means for a probable identification. If 1 c.c. of fresh or defibrinated blood is added to 6 c.c. of melted agar at 40° to 45° C., well shaken, inoculated with characteristic streptococci and poured in a Petri dish there will appear in twelve to twenty-four hours tiny colonies surrounded by clear zones of about $\frac{1}{4}$ to $\frac{1}{3}$ inch in diameter. Pneumococci and many varieties of streptococci, which occur together with characteristic forms in the throat, lungs and elsewhere, on the other hand produce only narrow zones of a green pigment. Anthony in our laboratory has found that from a streptococcus producing abundant hæmolytic substances strains may be obtained by selecting certain colonies which fail to make them. She has not been able to obtain from strains producing in first cultures the green pigment only any strains producing hæmolytic substances.

Duration of Life Outside of the Body.—This is not, as a rule, very

great. When dried in blood or pus, however, they may live for several months at room temperature, and longer in an ice-chest, and in gelatin and agar cultures they live for from one week to three months. In order to keep streptococci alive and virulent, it is best to transplant them frequently and to keep them in serum or ascitic fluid bouillon in small sealed glass tubes in the ice-chest.

Resistance to Heat and Chemicals.—The thermal death point of the streptococcus is between 52° and 54° C., the time of exposure being ten to twenty minutes.

Mercuric chloride, 1 : 5000; sulphate of copper, 1 : 200; trichloride of iodine, 1 : 750; peroxide of hydrogen, 1 : 50; carbolic acid, 1 : 100; cresol, 1 : 250; lysol, 1 : 300; creolin, 1 : 130, all kill streptococci within a few minutes.

Pathogenesis.—The majority of test animals are not very susceptible to infection by the streptococcus, and hence it is difficult to obtain any definite pathological alterations in their tissues through the inoculation into them of cultures of this organism by any of the methods ordinarily practised. White mice and rabbits, under similar conditions, are the most susceptible, and these animals are, therefore usually employed for experimentation. Streptococci, however, differ greatly in the effects which they produce in inoculated animals, according to their animal virulence, which is very different from human virulence. The most virulent when injected in the minutest quantity into the circulation or into the subcutaneous tissues of a mouse or rabbit, produce death by septicæmia. Those of somewhat less virulence produce the same result when injected in considerable quantities. Those still less pathogenic produce septicæmia, which is mild or severe, when injected into the circulation; but when injected subcutaneously, they produce abscess or erysipelas. The remaining streptococci, unless introduced in quantities of 20 c.c. or over, produce only a slight redness, or no reaction at all, when injected subcutaneously, and little or no effect when injected directly into the circulation. Many of the streptococci obtained from cases of cellulitis, abscess, empyema, and septicæmia belong to this group.

A number of varieties of streptococci have thus been discovered, differing in virulence and in their growth on artificial media; but all attempts to separate them into various classes, even with the use of specific serum, have largely failed, because the differences observed, though often marked, are not constant, many varieties having been found to lose their distinctive characteristics, and even to apparently change from one class to another. A further objection to any of the existing classifications of streptococci, which are based on the manner of growth on artificial culture media, is that it has been impossible to make any which would at the same time give even an approximate idea of their virulence. Experiments have proved that streptococci originally virulent may become non-virulent after long cultivation on artificial media, and, again, that they may return to their original properties after being passed through the bodies of

susceptible animals. The peculiar type of virulence which they may acquire tends to perpetuate itself, at least for a considerable time.

One important fact that experience teaches us is that those streptococci which are the most dangerous are those which have come immediately from septic conditions, and the more virulent the case the more virulent the streptococci are apt to be for animals of the same species. There seems also to be a strong tendency for a streptococcus to produce the same inflammation, when inoculated, as the one from which it was obtained; for example, streptococci from erysipelas tend to produce erysipelas, from septicæmia to produce septicæmia, etc. Streptococci, however, obtained from different sources (abscesses, puerperal fever, sepsis, erysipelas, etc.) are in many instances capable, under favorable conditions, of producing erysipelas when inoculated into the ear of a rabbit, as has been proved by experiment, provided they possess sufficient virulence.

Occurrence in Man.—Streptococci have been found to be the primary cause of infection in the following diseases: Erysipelas, circumscribed and extensive acute abscesses, impetigo, cellulitis (circumscribed as well as diffused), sepsis, puerperal infection, acute peritonitis, angina, bronchopneumonia, periostitis, osteomyelitis, synovitis, otitis media, mastoiditis, enteritis, irregular cases of rheumatic fever, meningitis, pleurisy, empyema, and endocarditis. Associated with other bacteria in diseases of which they were the specific cause, they have also been found as the secondary infection in many diseases, such as in pulmonary tuberculosis, bronchopneumonia, septic diphtheria, and diphtheritic scarlatina.

In cases of septic thrombus of the lateral sinus following mastoiditis there is almost certainly a streptococcus septicæmia. Libman has shown that an examination of the blood may be useful in diagnosis.

In diphtheritic false membranes this micrococcus is very commonly present, and is frequently the source of deeper infection, such as abscesses and septicæmia; and in certain cases attended with a diphtheritic exudation, in which the Loeffler bacillus has not been found by competent bacteriologists, it seems probable that the *Streptococcus pyogenes*, alone or with other pyogenic cocci, is responsible for the local inflammation and its results. These forms of so-called diphtheria, as first pointed out by Prudden, are most commonly associated with scarlatina and measles, erysipelas, and phlegmonous inflammation, or occur in individuals exposed to these or other infectious diseases. So uniformly are long-chained streptococci present in the pseudomembranes of patients sick with scarlet fever, that many investigators have suspected a special variety of them to be the cause of this disease. The same is true for smallpox. Many varieties are regularly found, however, in the throat secretion of healthy individuals (in 100 examinations by us we found long-chained streptococci in 83, and probably could have found them in some of the others by longer search). Their abundance in scarlet fever and smallpox is most probably due to their increase in the injured mucous membrane and entrance into the

circulation when the protective properties of the blood have been lowered.

Occurrence in Animals.—Besides streptococci similar to those in man, animals are infected by strains that are negative to Gram and fluidify gelatin. Udder infections of the cow and glandular diseases of the horse are frequently due to these. The streptococcic inflammations in animals are almost as frequent and serious as they are in man.

Effect on Tumors.—Fehleisen inoculated cultures, obtained in the first instance from the skin of patients with erysipelas, into patients in the hospital suffering from inoperable malignant growths—lupus, carcinoma, and sarcoma—and has obtained positive results, a typical erysipelatous inflammation having developed around the point of inoculation after a period of incubation of from fifteen to sixty hours. This was attended with chilly sensations and an elevation of temperature. Persons who had recently recovered from an attack of erysipelas frequently proved to be immune. These experiments were undertaken on the ground that malignant tumors had previously been found to improve or entirely disappear in persons who had recovered from accidental erysipelas. During the last few years this fact has been therapeutically applied to the treatment of malignant tumors by the artificial production of erysipelas by the inoculation of pure cultures of streptococcus or of their toxic products. Lately the mixed toxins of the streptococcus and B. prodigiosus have been given, and it now appears as if the toxins of the latter organism were much the more important.

Results from Injections in Tumors.—In some cases of sarcoma this method has met with considerable success; in carcinoma, however, the results have been very slight. In this country the experimental work upon this subject and the actual treatment of cases have been largely carried out by or under the direction of Coley. He has kindly sent me the following notes on his results:

"The improvement and inhibitory action which the toxins have upon carcinoma have proved to be, in nearly all cases, but temporary.

"On the other hand, in sarcoma, which is the only form of malignant tumor in which I have advocated the treatment, sufficient time has elapsed to enable us to draw the following conclusions:

"The toxins injected subcutaneously into the tissues, either into the tumor substance or into parts remote from the tumor, exercise a distinctly inhibitory action upon the growth of nearly all varieties of sarcoma. This action is the least marked in melanotic sarcoma, and thus far no cases of this form of tumor have disappeared under the treatment. The influence of the toxins upon round-celled sarcoma is much more powerful than it is upon melanotic, although distinctly less than upon the spindle-celled variety. A number of cases of round-celled sarcoma in which the diagnosis was questioned disappeared, and the patients have remained well beyond three years. Nearly half of the cases treated show no appreciable decrease in

size; the majority of the others which did show marked improvement at first, after decreasing in size for a few weeks, again began to increase and were no longer influenced by the treatment.

"In half of the cases of spindle-celled sarcoma treated by the toxins the disease had disappeared entirely, and the majority of the successful cases have remained well sufficiently long to justify their being regarded as cured. It should be distinctly stated that all of the tumors under consideration were inoperable, as I have never advised treatment except in such cases.

"I have now a number of cases of spindle-celled sarcoma which have remained well beyond three years; one case of mixed (round and spindle) celled, after remaining well three years, had a return in the abdomen, and died about eight months later. The result here certainly establishes the correctness of the early diagnosis."

Some surgeons have not had nearly as favorable results as Coley. I think there is no question that in a small percentage of cases good results have been obtained.

Production of Toxic Substances.—There is no doubt that the streptococcus causes fever, general symptoms of intoxication, and death by means of toxic substances which it forms in its growth; but we know very little about these substances or how they are produced. The cell substance of streptococci possesses only slight toxicity. Ruediger[1] has shown that a specific streptolysin is formed which produces a true antibody. The poisons while partly extracellular are mostly contained in the cell substance. Heat destroys a portion of them. They appear to attack especially the red blood cells, and this hæmolytic action seems to be to some degree in proportion to the virulence of the organism.

Susceptibility to Streptococcus Infection.—As with the ever-present staphylococci, whose virulence, as we have seen, is usually slight, the streptococci are more likely to invade the tissues, forming abscesses or erysipelatous and phlegmonous inflammation in man when the standard of health is reduced from any cause, and especially when by absorption or retention various toxic organic products are present in the body in excess. It is thus that the liability to these local infections, as complications of operations or sequelæ of various specific infectious diseases, in the victims of chronic alcoholism, and constitutional affections, etc., are to be explained. It seems established that the absorption of toxic products formed in the alimentary canal as a result of the ingestion of improper food, or in consequence of abnormal fermentative changes in the contents of the intestine, or from constipation predispose to infection.

Immunity.—In none of the streptococcus inflammations do we notice much apparent tendency to the production of immunizing and curative substances in the blood by a single infection.

Severe general infections usually progress to a fatal termination after a few days, weeks, or months. It is true, however, that cases of erysipelas, cellulitis, and abscess, after periods varying from a few

[1] Ruediger. Jour. Amer. Med. Assn., 1903, xli., page 962.

days to months, tend to recover, and to a certain extent, therefore, we may assume that protective agents have been produced. In these cases, however, we know from experience that faulty treatment, by lessening the local or general resistance, would, as a rule, cause the subsiding infection again to progress perhaps even to a more serious extent than the original attack. Koch and Petruschky tried a most interesting experiment. They inoculated cutaneously a man suffering from a malignant tumor with a streptococcus obtained from erysipelas. He developed a moderately severe attack, which lasted about ten days. On its subsidence they reinoculated him; a new attack developed, which ran the same course and over the same area. This was repeated ten times with the same results.

This experiment proved that in this case, at least, little if any lasting curative or immunizing substances were produced by repeated attacks of erysipelas, and that the recovery from each attack was due to local and transitory protective developments.

The severe forms of infection, such as septicæmia following injuries, operations, and puerperal infections, show little tendency to be arrested after being well established. Having, then, in remembrance, the above facts, let us consider the results already obtained in the experimental immunization and treatment of animals and men suffering from or in danger of infection with streptococci. Knorr succeeded in producing a moderate immunity in rabbits against an intensely virulent streptococcus by injections of very slightly virulent cultures. Marmorek was the first to attempt the production of a curative serum on a large scale.

Influence of Serum from Immunized Animals upon Streptococcus Infections in Other Animals.—In the table are given the results following the injection of small amounts of a serum which represents in immunizing value what about one-third of the horses are able to produce when given in gradually increasing doses the living, virulent streptococcus. In the following experiments the serum and culture were injected subcutaneously in rabbits at the same time, but in opposite sides of the body:

TABLE—*Showing Strength of Average Grade of Antistreptococcic Serum given by Selected Horses after Six Months of Injection of Suitable Amounts of Living Streptococci.*

	Weight of rabbit	Amounts inoculated		Results	Autopsy
Serum and culture:	Grms.	Serum	Cult.		
1. Inoculated together	1430	0.25 c.c.	0.01 c.c.	Lived	
2. Inoculated together	1350	0.125 c.c.	0.01 c.c.	Lived	
3. On opposite sides	1770	0.1 c.c.	0.01 c.c.	Lived	
4. On opposite sides	1630	0.1 c.c.	0.01 c.c.	Lived	
Controls:					
1. Rabbits injected with culture only	1750	.....	0.001 c.c.	Died in 4 days	Streptococci infection.
2. Rabbits injected with culture only	1870	.....	0.001 c.c.	Died in 24 hrs.	Streptococcic infection.

The above results have been repeatedly obtained, and are absolutely conclusive that the serum of properly selected animals, which have been repeatedly injected with living streptococci in suitable doses possesses bactericidal properties upon the same streptococcus when it comes in contact with it within the bodies of animals.

Definite protection from the serum has been obtained by many reliable observers since Marmorek's first reports.

Is Protection Afforded by the Same Serum against all Varieties of Streptococci?—We have tested the protective value of one serum against streptococci derived from five different sources. First, the streptococcus given us by Marmorek, which was obtained from a case of angina. Its virulence is now such, after having passed through hundreds of rabbits, that 0.000001 c.c. is the average fatal dose. Second, a streptococcus obtained from a case of erysipelas in England. Its virulence is 0.00001 c.c. on the average. Third, a streptococcus obtained from a case of cellulitis, its virulence being about 6 c.c. Fourth, a streptococcus sent me by Theobald Smith. Its virulence is such that 0.1 c.c. is the average fatal dose. Fifth, another culture sent me by Smith, which grew in short chains and was obtained from milk; its virulence was similar to No. 4.

Against the first three streptococci derived from three different varieties of infection existing in three different countries the serum produced by the streptococcus from England had nearly the same value. Against the latter two streptococci, as well as against a streptococcus from a case of endocarditis, which resembled in some respects the pneumococci, the serum had no effect.

The results of numerous investigators indicate that the majority but not all of streptococci met with in cellulitis, erysipelas, and abscess will be influenced by the same serum. Those obtained from cases of pneumonia and endocarditis and other exceptional infections are apt to have individual characteristics.

Polyvalent Serum.—In order that the serum may have specific anti-bodies for the variety of streptococci causing each separate infection each horse is now injected with a large number of different varieties of streptococci. This serum will not be quite as efficient as if made by the streptococcus infecting the treated case, but will be fairly efficient for all cases.

Preparation of the Serum.—Antistreptococcus serum is obtained from the horse after treatment by repeated injections of living or dead streptococcus cultures derived from human sources. As a rule, a number of varieties are given at the same time so that the serum will be active against any variety causing the infection. If the serum is to be used in scarlet fever, the streptococci used should be from cases of scarlet fever. The procuring of a serum of the highest potency requires a considerable number of animals, for some produce with the same treatment a more protective serum than others. The serum must be sterile from streptococcus as well as from other contaminations.

Stability of the Serum.—It is fairly stable but, after several months, the serum loses some of its protective value. It should be kept in a cool and dark place.

Standardization of the Value of the Serum.—There is at present no satisfactory way. The value of the serum is sometimes measured by the amount required to protect against a multiple of a fatal dose of a very virulent streptococcus of the same type as the one used to inject the horses. The dose is usually a thousand times the average fatal amount of a very virulent streptococcus.

Other methods of standardization, such as the estimation of the amount of opsonins or agglutinins present, are also used.

Therapeutic Results.—To estimate the exact present and future value of antistreptococcus serum is a matter of the utmost difficulty. Many of the cases reported are of little or no help, because, no cultures having been made, we are in doubt as to the nature of the bacterial infection.

In the cases of puerperal fever, erysipelas, and wound infection that we have seen, the apparent results under the treatment have not been uniform. We have frequently observed favorable results which appeared to be due to the serum when doses of 50 to 60 c.c. were given intravenously.

In a number of cases of septicæmia where for days chills had occurred daily they ceased absolutely or lessened under daily doses of 20 to 50 c.c. The temperature, though ceasing to rise to such heights, did not average more than one or two degrees lower than before the injections. In some cases the serum treatment was kept up for four weeks. Some cases convalesced; others after a week or more grew worse and died. In some cases the temperature fell immediately upon giving the first injection of serum, and after subsequent injections remained normal, and the cases seemed greatly benefited. As a rule, in these cases no streptococci or any other organisms were obtained from the blood. In bronchopneumonia, laryngeal diphtheria, tonsillitis, smallpox, and phthisis, we have seen little effect.

The results obtained here in New York by both physicians and surgeons have not, on the whole, been very encouraging.

In some of the cases where apparently favorable results were obtained other bacteria than streptococci were found to be the cause of the disease. We believe that the following conclusions will be found fairly accurate:

The serum will in animals limit an infection already started if it has not progressed too far. The apparent therapeutic results in cases of human streptococcus infection are variable. In some cases the disease has undoubtedly advanced in spite of large injections, and here it has not seemed to have had any effect. In other cases good observers rightly or wrongly believe they have noticed great improvement from it. Except rashes, few have noticed deleterious results, although very large doses have been followed in several instances, for a short time, by albuminous urine.

In suitable cases we are warranted, we believe, in trying it, but we should not expect very striking results.

For our own satisfaction, and to increase our knowledge, we should always have satisfactory cultures made when possible, and the streptococci, if obtained, tested with the serum used in the treatment. In the cases where we want most to use the serum, such as puerperal fever, septicæmia, ulcerative endocarditis, etc., we find that it is very difficult to make a bacteriological diagnosis from the symptoms, and in over one-half of the cases even the bacteriological examination carried out in the most thorough way will fail to detect the special variety of bacteria causing the infection. This is often a great hindrance to the proper use of curative antistreptococcic serum, for it, of course, has no specific effect upon the course of any infection except that due to the streptococcus and the full effect only on its own type.

Care should be taken to get the most reliable serum; much on the market is worthless, and as it is weak, and the testing for strength is difficult or impossible, full doses (30–50 c.c.) of serum should be given if the case is at all serious, for the dose is limited only by the amount of horse serum which we feel it safe to give, not because we have given sufficient protective substance. Intravenous injections give better results than those given subcutaneously. Studdiford has obtained good results by adding to the intravenous injection the packing of the septic uterus with gauze impregnated with the serum.

Scarlet Fever.—In Vienna for some years the serum of horses treated at each injection with a number of strains of streptococci derived from scarlet fever cases has been used in this disease. The serum given in large doses of 100 to 200 c.c. has apparently given good results in about half of those treated. It is only used in severe cases. Moser has chiefly advocated its use. One of us had the opportunity to look over the histories of his cases. Although left in doubt as to its value, it appears to us as worth a trial. Our own results in thirty cases have been rather favorable.

Bacteriological Diagnosis.—Streptococci, using the name in a broad sense, can often be demonstrated microscopically by simply making a smear preparation of the suspected material and staining with methylene-blue solution or diluted Ziehl's fluid. In order to demonstrate them microscopically in the tissues, the sections are best stained by Kühne's methylene-blue method. In all cases, even when the microscopic examination fails, the cocci may be found by the culture method on plate agar or slanted agar at 37° C. To obtain them from a case of erysipelas it is best to excise a small piece of skin from the margin of the erysipelatous area in which the cocci are most numerous; this is crushed up and part of it transferred to ascitic or serum bouillon, and part is streaked across freshly solidified agar in a Petri dish on which a drop of sterile rabbit's blood had previously been placed. Both are kept in the incubator at 37° C.

In septicæmia the culture method is always required to demonstrate the presence of streptococci, as the microscopic examination of

specimens of blood is not sufficient. For this purpose from 10 to 15 c.c. of the blood should be drawn from the vein of the arm aseptically by means of a hypodermic needle, and to each of three tubes containing 10 c.c. of melted nutrient agar kept at about 43° C. 1 c.c. of blood is added. After thoroughly mixing the contents are poured into Petri dishes. The remainder is added to several flasks containing 250 c.c. of nutrient broth, in order to produce a development of the cocci, which are found in small numbers in the blood. Petruschky is of the opinion that the cocci can be best shown in blood by animal inoculation. Having withdrawn from the patient 10 c.c. of blood by means of a hypodermic syringe, under aseptic precautions, he injects a portion of this into the abdominal cavity of a mouse, while the other portion is planted in bouillon. Mice thus inoculated die from septicæmia when virulent streptococci are present in only very small numbers in the blood. If a successful inoculation takes place we can, through the absence or presence of the development of capsules, often differentiate between the pneumococcus and the streptococcus, which cultures may fail to do. The development of a wide, clear zone about the colonies (upon blood-agar), without a development of green pigment, indicates that the streptococci belong to the pyogenes type. The absence of a definite zone and the development of a green color indicates that they are pneumococci, or streptococci which in these two respects resemble pneumococci. The growth in the Hiss inulin serum medium will generally differentiate between the two, as the pneumococci usually coagulate the serum, while the great majority of streptococci do not. The morphological and cultural characteristics of the streptococcus give us, unfortunately, no absolute knowledge as to the influence which the protecting serum will have. The actual test is here our only method. The detection of the streptococcus in the blood is in itself an unfavorable prognostic sign.

The blood cultures in many cases of supposed septicæmia give no results, for many of these cases develop their symptoms and even die from the absorption of toxins from the local infection, such as an amputation wound or an infected uterus or peritoneum, and the bacteria never invade the blood. When we get negative results we are, as a rule, utterly unable to test the case with curative serums with any accuracy, for the sepsis may be due to either the streptococcus, colon bacillus, staphylococcus, or a number of other pathogenic varieties of bacteria.

CHAPTER XXVII.

THE DIPLOCOCCUS OF PNEUMONIA (PNEUMOCOCCUS, STREPTOCOCCUS PNEUMONIÆ, MICROCOCCUS LANCEOLATUS).

THE DIPLOCOCCUS OF PNEUMONIA.

THE diplococcus of pneumonia was observed in 1880 almost simultaneously by Sternberg and Pasteur in the blood of rabbits inoculated with human saliva. In the next few years Talamon, Friedländer, A. Fraenkel, Weichselbaum, and others subjected this microorganism to an extended series of investigations and proved it to be the chief etiological factor in the production of lobar or croupous pneumonia in man.

The outcome of the various investigations proved that the acute lung inflammations, especially when not of the frank lobar pneumonia type, are not excited by a single variety of microörganism, and that the bacteria involved in the production of pneumonias are also met with in inflammations of other tissues.

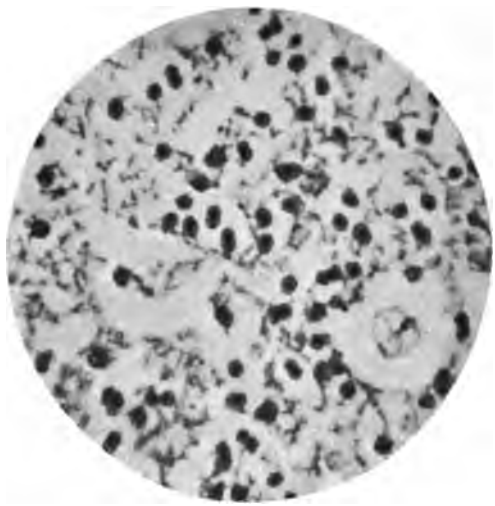
In any individual pneumonic inflammation it is also found that more than one variety of bacteria may be active, either from the start or as a later addition to the original primary infection.

Among all the microörganisms active in exciting pneumonia, the diplococcus of pneumonia is by far the most common, being almost always present in primary lobar pneumonia and as frequently as any other germ in acute bronchopneumonia and metastatic forms. Besides the different varieties of pneumococci the following bacteria are capable of exciting pneumonia: *Streptococcus pyogenes, Staphylococcus pyogenes, Bacillus pneumoniæ, Bacillus influenzæ, Bacillus pestis, Bacillus diphtheriæ, Bacillus typhi, Bacillus coli,* and the *Bacillus tuberculosis.* Since the varieties of bacteria exciting acute pneumonia, with the exception of the pneumococcus, are met with more frequently in other inflammations and have been described elsewhere, they will only be noticed in this chapter so far as their relation to pneumonia demand.

Morphology.—Typically, the pneumococcus occurs as spherical or oval cocci, usually united in pairs, but sometimes in longer or shorter chains consisting of from three to six or more elements and resembling the streptococcus. The cells, as they commonly occur in pairs, are somewhat oval in shape, being usually pointed at one end—hence the name *lanceolatus* or lancet-shaped. When thus united the junction, as a rule, is between the broad ends of the oval, with the pointed ends turned outward; but variation in form and arrangement of the cells is characteristic of this organism, there being great differences

according to the source from which it is obtained. As observed in the sputum and blood it is usually in pairs of lancet-shaped elements, which are surrounded by a capsule. (See Fig. 123.) When grown in fluid culture media longer or shorter chains are frequently formed, which can scarcely be distinguished from chains of certain streptococci, except that, as a rule, the length of the chain is less and the pairs of diplococci are farther apart. In cultures the individual

FIG. 123



Diplococcus of pneumonia from blood, with surrounding capsule stained by method of Hiss.

cells are almost spherical in shape, and except in certain varieties are rarely surrounded by a capsule. (See Fig. 124.) The pneumococcus is by some classed as a streptococcus.

The capsule is best seen in stained preparations from the blood and exudates of fibrinous pneumonia or from the blood of an inoculated animal, especially the mouse, in which it is commonly, though not always, present. It is seldom seen in preparations from cultures unless special media are employed. Flagella are not present.

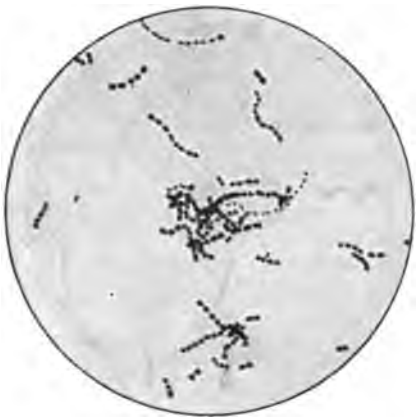
Staining.—It *stains* readily with ordinary aniline colors; it is not decolorized after staining by Gram's method. The capsule may be demonstrated in blood or sputum either by Gram's or Welch's (glacial acetic acid) method, or the copper sulphate method of Hiss.

Biology.—It grows equally well with or without oxygen; its parastic nature is exhibited by the short range of temperature at which it usually grows—viz., from 25° to 42° C.—best at 37° C. In the cultivation of this organism neutral or slightly alkaline media should be employed. The organism grows feebly on the serum-free culture media ordinarily employed for the cultivation of bacteria—viz., on nutrient agar and gelatin, in bouillon. The best medium for its growth is a mixture of one-third human or animal blood serum or ascitic or pleuritic

fluid and two-thirds bouillon, or nutrient agar streaked with human or rabbit blood.

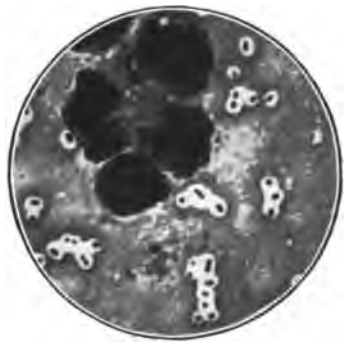
Growth on Agar.—Cultivated on plain nutrient agar, after twenty-four to forty-eight hours at 37° C., the deep colonies are hardly visible to the eye. Under the microscope they appear light yellow or brown in color and finely granular. The surface colonies are large, equalling in size those of streptococci, but are usually more transparent. If blood serum or ascitic fluid be added to the agar the individual colonies are larger and closer together, and the growth is more distinct in consequence and of a grayish color. The surface colonies are almost circular in shape under a magnification of 60 diameters, finely granular in

FIG. 124



Pneumococcus from bouillon culture, resembling streptococcus.

FIG. 125



Pneumococci in peritoneal pus. Stained with fuchsin. ×1000 diameters. Clear spaces indicate capsules.

structure, and may have a somewhat darker, more compact centre, surrounded by a paler marginal zone. With high magnification cocci in twos and short rows often distinctly separated are seen at the edges.

Growth on Blood Serum.—The growth on Loeffler's blood-serum mixture is very similar to that on agar, but somewhat more vigorous and characteristic, appearing on the surface as a delicate layer of dew-like drops.

Growth in Bouillon.—In bouillon, at the end of twelve to twenty-four hours in the incubator, a slight cloudiness of the liquid will be found to have been produced. On microscopic examination cocci can be seen to be arranged in pairs or longer or shorter chains. After one or two transplantations the pneumococci frequently fail to grow.

Growth in Milk.—It grows readily in milk, causing coagulation with the production of acid, though this is not constant with some forms intermediate between the streptococcus and pneumococcus.

Growth on Gelatin.—The growth on gelatin is slow, if there is any development at all, owing to the low temperature—viz., 24° to 27° C.—above which even the most heat-resistant gelatin will melt. The gelatin is not liquefied.

Special Media.—When cultures are grown on serum-free media the vitality of some cultures may indeed be indefinitely prolonged; but after transplantation through several generations it is found that the cultures begin to lose in virulence, and that they finally become non-virulent. In order to restore this virulence, or to keep it from becoming attenuated, it is necessary to interrupt the transplantation and pass the organism through the bodies of susceptible animals.

With the view of overcoming these obstacles in the way of cultivating this micrococcus, several special media have been proposed by various experimenters in the place of the ordinary culture media. The best fluid medium for the growth of the pneumococcus is Marmorek's mixture, consisting of bouillon 2 parts and ascitic or pleuritic fluid 1 part. In this fluid pneumococci grow well, and cultures when preserved in a cool place and prevented from drying retain their vitality and also their virulence for a number of weeks. Lambert has found cultures in this medium alive and fully virulent after eight months.

Loeffler's blood-serum mixture is a good, solid tube medium for making cultures, and is very convenient and useful at autopsies. One and one-half per cent. fluid nutrient agar mixed with one-third its quantity of warm ascitic fluid makes an excellent plate medium.

Nutrient agar, with fresh rabbit blood smeared over it makes an excellent medium for growth, but prevents the development of agglutinable substance. On this medium, in a moist atmosphere at 36° C., the cultures retain their viability and virulence for rabbits for months.[1]

Hiss Serum Media with and without Inulin.—These are very useful. The inulin is fermented by typical pneumococci with coagulation of the serum, while most streptococci fail to ferment the inulin. This medium is, therefore, of considerable diagnostic value.

Calcium Broth with or without Dextrose.—This medium has proven of great value for the propagation of cultures where agglutination tests are to be carried out. The addition of a small piece of marble to each tube of broth is the most satisfactory way of preparing it. Marble broth for this purpose was suggested independently by Bolduan and Hiss.

Resistance to Light, Drying, and Germicidal Agents.—On artificial culture media the pneumococci tend to die rapidly. This is partially due to the acid produced by their growth. In sputum they live much longer.

Pneumonic sputum attached in masses to clothes, when dried in the air and exposed to diffuse daylight, retains its virulence, as shown by injection in rabbits, for a period of nineteen to fifty-five days. Exposed to direct sunlight the same material retains its virulence after but a few hours' exposure. This retention of virulence for so long a time under these circumstances is accounted for by the protective influence afforded by the dried mucoid material in which the micrococci were embedded. Guarnieri observed that the blood of inoculated animals, when rapidly dried in a desiccator, retained its virulence for months; and Foá found that fresh rabbit blood, after inoculation and cultivation in the incubator for twenty-four

[1] The green color produced by all pneumococci in blood-agar media, and showing especially well in poured blood-agar plates is not diagnostic of this organism, as some strains of streptococci produce just as intense a green.

hours, when removed at once to a cool, dark place, retained its virulence for sixty days. There are many conditions, therefore, in which the virulence of the micrococcus is retained for a considerable length of time. To germicidal agents pneumococci are very sensitive. The fine spray expelled in coughing and loud speaking that remains suspended in the air soon dries so completely that no pneumococci survive after two hours.

Attenuation of Virulence.—This may be produced in various ways. The loss of virulence which occurs when the micrococcus is transplanted through several generations in culture fluid containing no blood has already been referred to. An apparent attenuation of virulence takes place also spontaneously in the course of pneumonia. It has been shown by daily puncture of the lung in different stages of the pneumonic process that the virulence of the organism diminishes as the disease progresses, and that the nearer the crisis is approached the more attenuated it becomes. This attenuation is probably only apparent. So many more microörganisms are living in each cubic centimetre of fluid during the early stages of a pneumonia that much smaller quantities kill. If a little sputum be taken at different periods in the disease and planted in ascitic bouillon the resultant cultures will not vary greatly in virulence. The average virulence for rabbits of cultures made from pneumonic sputum is greater than in those from normal sputum.

Restoration and Increase of Virulence.—The simplest and perhaps the most reliable method of restoring lost virulence for any susceptible animal is by passage through the bodies of highly susceptible animals of the same species. Growth in fresh blood also increases it for the homologous animal.

Toxin Production.—We have little exact knowledge upon the nature of the substances produced by or through the growth of the pneumococci in animal tissues or artificial media. Rosenow[1] showed that the autolysis of virulent pneumococci in NaCl solution brings into the solution a group of substances which inhibits the action of the pneumococco-opsonin.

Occurrence in Man during Health.—It is probable that in crowded communities the pneumococcus is present on the mucous membranes of most persons. We have found it generally present not only in the throats of persons living in New York City, but also in those of persons living on farms and in the Adirondack Mountains. It is commonly present only on the mucous membranes of the bronchi, trachea, pharynx, and nostrils. The healthy lung seems to be generally free from it. Judging from animal tests it is very possible that the virulence for man of the organisms present in health is much less than the virulence of those in a pneumonic lung.

Pathogenicity in Man.—Characteristic or atypical pneumococci are present in fully 95 per cent. of characteristic cases of lobar pneumonia. Usually no other bacteria are obtained from the lungs.

[1] Rosenow. Jour. Infect. Dis., 1907, iv., p. 285.

Atypical cases usually show the same conditions, but they may be due to streptococci, influenza bacilli, etc. The more recent the infection the greater is the number of bacteria found in the diseased lung area. As the disease progresses these decrease in number until finally at the crisis they disappear from the tissues, though at this time and long after convalescence they may be present in the sputum. In atypical forms of pneumonia they may remain longer in the tissues, and in walking pneumonia they may be absent in the original centres of infection or present only as attenuated varieties, while the surrounding, newly formed foci may contain fully virulent cocci. It has been shown by Netter that more than one-half of the cases of bronchopneumonia, whether primary or secondary to some other disease, as measles and diphtheria, both in children and adults, are due to the diplococcus of pneumonia. Others, such as Pearce, have found other microörganisms, especially the streptococci, in the majority of cases. These findings will be considered at the end of the chapter.

The pneumococci are found partly in the alveoli and bronchioles of the inflamed lung and partly in the lymph channels and blood capillaries. Most of the organisms are found free, but a few are found in the leukocytes. Through the lymph channels they find their way to the pleura and to adjacent lymph glands. From the capillaries they find their way to the general blood current, and thus to distant parts of the body. In about 20 per cent. of cases the pneumococci are so abundant that they can be found in cultures made from 5 to 10 c.c. of blood. In a number of instances the fœtus has been found infected. The pneumococci are also responsible for:

Inflammations Complicating Pneumonia.—In every case of lobar pneumonia and in most cases of bronchopneumonia pleurisy is developed, which is excited by the same microörganism that was predominant in the pneumonia. With pneumococci the exudate is usually moderate and of a fibrinous character, but may be more abundant and of a serofibrinous or purulent character. When the pleurisy is marked it is more apt to continue after the cessation of the pneumonia. Pleurisy due to pneumococci is more apt to go on to spontaneous recovery than that due to streptococci or staphylococci.

The most frequent pneumococcic infections next to pleurisy, following a pneumonia, are those of the middle ear, pericardium, endocardium, and meninges, and these not infrequently arise together. Pneumococcic inflammations of the heart valves are apt to be followed by extensive necrosis and growth of vegetations. In these cases pneumococci can sometimes be found in the blood for many weeks. Pericarditis due to pneumococci is a frequent complication, but is usually very slightly developed. Meningitis due to pneumococci may be either fibrinous or purulent or both and is apt to be secondary to otitis, mastoiditis, or pneumonia. Arthritis, periarthritis, and osteomyelitis are rarer complications of a pneumococcic pneumonia. Besides moderate parenchymatous inflammation of the kidney, which occurs in most cases of pneumonia, well-marked inflam-

mation may occur in which pneumococci exist in the kidney tissues in large numbers.

How is the pneumococcus conveyed from its original seat in the lungs to distant internal organs? Chiefly by means of the blood vessels and lymphatics, in both of which it has been found in great numbers. Proof enough of its conveyance through the lymphatics is afforded by the frequent occurrence of inflammations of the serous membranes complicating pneumonia; but two cases in particular have been reported by Thue of pleurisy and pericarditis following pneumonia in which the lymph capillaries have been found to be filled with diplococci, as if injected. Their presence in the blood after death has been amply proved by numerous investigations. In many instances they have been recovered from the blood during life. Lambert, as a rule, found them in all fatal cases twenty-four to forty-eight hours before death. This examination has considerable prognostic value, as nearly all cases in which the pneumococcus is found end fatally. This micrococcus has been shown experimentally to be capable of producing various forms of septicæmia—local phlegmonous inflammations, peritonitis, pleuritis, and meningitis. A further proof of the transmission of this organism by means of the blood is given by Foá and Bordoni-Uffreduzzi in their investigations into intrauterine infection in pneumonia and meningitis. These investigators have demonstrated the presence of the micrococcus lanceolatus in fetal and placental blood and in the uterine sinuses in maternal pneumonia. There being no question, therefore, as to the possibility of the conveyance of the infective agent by means of the blood and the lymph to all parts of the body, we need not wonder at the multiplicity of the affections complicating a pneumonia, which are caused by this micrococcus; and not only the secondary, but also the primary diseases, as of the brain and meninges, may be explained in the same way. Knowing that the saliva and nasal secretions under normal conditions so frequently afford a resting place for the micrococci, we have only to assume the production of a suitable culture medium for these parasites in the body, brought about by an abnormal condition of the mucous membranes from exposure to cold, or a reduction of the vital resisting power of the tissue cells in any of the internal organs, caused by disease, traumatism, excess of various kinds, etc., to comprehend readily how an individual may become infected with pneumococci, either primarily affecting the lungs and secondarily other organs in the body, or primarily attacking the middle ear, the pericardial sac, the pleura, the serous cavities of the brain, etc.

Presence in Inflammatory Process Not Secondary to Pneumonia.—It is now known that the pneumococcus may infect and excite diseases in many tissues of the body independent of any preliminary localization in the lung. As a rule, these processes are acute and usually run a shorter and more favorable course than similar inflammations due to the streptococci.

The most frequent primary lesions excited by the pneumococcus

after lobar pneumonia, bronchopneumonia, and bronchitis are probably meningitis, otitis media with its complicating mastoiditis, endocarditis, pericarditis, rhinitis, tonsillitis, conjunctivitis, and keratitis; septicæmia, arthritis, and osteomyelitis; inflammations of the epididymis, testicles, and Fallopian tubes; peritonitis, etc.

Pneumococcic peritonitis and appendicitis are not so very frequent. The exudate is usually seropurulent.

Conjunctivitis due to pneumococci frequently occurs in epidemic form and is frequently associated with rhinitis.

From statistics collected by Netter the following percentages of diseases were caused by the pneumococcus:

Pneumonia	65.9 per cent. in adults.
Bronchopneumonia	15.8 per cent. in adults.
Meningitis	13.0 per cent. in adults.
Empyema	8.5 per cent. in adults.
Otitis media	2.4 per cent. in adults.
Endocarditis	1.2 per cent. in adults.

In 46 consecutive pneumococcus infections in children there were:

Otitis media	29 cases.
Bronchopneumonia	12 cases.
Meningitis	2 cases.
Pneumonia	1 case.
Pleurisy	1 case.
Pericarditis	1 case.

The pneumococcus and streptococcus are the two most frequent organisms found in otitis media. The cases due to the pneumococcus are apt to run the shorter course, but have a tendency to spread to the meninges and cause a meningitis. The pneumococci may also find their way into the blood current. This usually follows after sinus thrombosis.

In bronchitis the pneumococcus is frequently met with alone or in combination with the streptococcus, the influenza bacillus, or other bacteria.

In certain epidemics pneumococcic bronchitis and pneumonia simulate influenza very closely and cannot be differentiated except by bacteriological examinations.

Primary pneumococcic pleurisy is frequent in children: it is very often purulent, but may be serous or serofibrinous. Its prognosis is better than that in cases due to other organisms. Frequently we have streptococci and staphylococci associated with the pneumococci.

Pathogenesis in Lower Animals.—Most strains of the *Micrococcus lanceolatus* are moderately pathogenic for numerous animals; mice and rabbits are the most susceptible, indeed some stains are intensely virulent for these animals, while guinea-pigs and rats are much less susceptible. Pigeons and chickens are refractory. In mice and rabbits the subcutaneous injection of small or moderate quantities of pneumonic sputum in the early stages of the disease, or of a twenty-four-hour ascitic broth culture from such sputum, or of a pure, virulent ascitic broth culture of the micrococcus, usually results in the death

of these animals in from twenty-four to forty-eight hours. The course of the disease produced and the post-mortem appearances indicate that it is a form of septicæmia—what is known as sputum septicæmia. After injection there is loss of appetite and great debility, and the animal usually dies some time during the second day after inoculation. The post-mortem examination shows a local reaction, which may be of a serous, fibrinous, hemorrhagic, necrotic, or purulent character; or there may be combinations of all of these conditions. The blood of inoculated animals immediately after death often contains the micrococci in very large numbers. For microscopic examination they may be obtained from the blood, and usually from pleural and peritoneal exudations when these are present.

True localized pneumonia does not usually result from subcutaneous injections into susceptible animals, but injections made through the thoracic walls into the substance of the lung may induce a typical fibrinous pneumonia. This was first demonstrated by Talamon, who injected the fibrinous exudate of croupous pneumonia, obtained after death or drawn during life from the hepatized portions of the lung, into the lungs of rabbits. Wadsworth showed that by injecting virulent pneumococci into the lungs of rabbits which had been immunized, a typical lobar pneumonia was excited, the bactericidal property of the blood being sufficient to prevent the general invasion of the bacteria.

Varieties of the Pneumococcus.—As among all other microörganisms minutely studied, different strains of pneumococci show quite a wide range of variation in morphology and virulence. Some of the variations are so marked and so constant that they make it necessary to recognize several distinct varieties of the pneumococcus, and to class as pneumococci certain varieties which have before this been classed as streptococci—*e. g.*, the so-called *Streptococcus mucosus capsulatus* (*Streptococcus mucosus* Schottmüller), when first isolated from pneumonic exudate or elsewhere, and planted on artificial culture media containing serum, grows as a rounded coccus with a small dense distinct capsule, principally in short or medium chains; it produces a large amount of mucus-like zooglia, forming very large spreading colonies; it promptly coagulates fluid serum media containing inulin. It is also very virulent for mice, but only moderately virulent for rabbits. After a number of culture generations on ordinary nutrient agar it apparently loses most of these characteristics. It then grows in small colonies principally as naked diplococci which may be elongated and pointed, produces no zooglia, and loses most of its virulence for mice and rabbits. It still coagulates inulin serum media, and when transferred to serum media regains its former morphological characteristics. For these reasons we consider this organism a distinct variety of the pneumococcus. This variety of pneumococcus has been isolated by us from the lungs after death following lobar pneumonia, out of twenty consecutive autopsies, as the only organism present twice, and with another variety of pneumococcus once. Together with

other varieties it was isolated from four out of twenty specimens of pneumonic sputum, and from sixty specimens of normal throat secretion five times.

Another group of pneumococci quite constantly produces large forms and large capsules. Still another group produces principally small forms and small capsules. Another group might be made of morphologically typical pneumococci which do not coagulate inulin serum media.

Immunity.—Following an attack of pneumonia some immunity is established, but this lasts but a short time. Early in the history of this organism experiments were begun for the production of immunity in animals by means of preventive inoculations. Later it was found that after successive injections of gradually increasing doses of virulent pneumococci into certain animals (horse, sheep, goat, rabbit), a serum of some protective and curative power in experimental animals was obtained. The mode of action of this serum is still the subject of study. According to Wright, Neufeld, and others, its activity is due to the presence of certain substances called opsonins (Wright), or bacteriotropic substances (Neufeld), which act on the bacteria in such a way as to prepare them for ingestion by the phagocytes. The extent to which phagocytosis brings about the crisis and healing in pneumonia is not known.

Agglutination Reactions.—Neufeld, Clairmont, and others demonstrated agglutinating substances for the pneumococcus in the blood of immunized animals; they concluded, from their observations, that this test might be used as a means of diagnosis. The low index obtained by them and the few strains used seemed to justify this assumption.

We have found, however, in our laboratory (Collins) that when a high index is reached or a large number of strains tested, the variability of the reaction is so great as to render it impractical as a means of diagnosis.

For instance, one strain may produce agglutinins common to itself and four or five other strains, while 70 or 80 other strains (all being typical pneumococci) will fail to react in the serum.

This diversity of reaction is confirmed also by the absorption tests.

In the case of the pneumococcus mucosus (*Streptococcus mucosus* Schottmüller) Collins found greater uniformity of reaction, all strains tested reacting alike, and the agglutinins of one member of the group were absorbed by the other members.

Therapeutic Experiments.—The number of cases reported in which the blood serum of animals artificially immunized against pneumonic infection has been used for the treatment of the disease in human beings, although numerous, has not led to the formation of a definite opinion as to the final value of this as a therapeutic agent. In the cases we have observed there has been, in some a slight immediate lowering of the temperature, in others no apparent change. As a rule, the cases did rather better than was expected, but certainly no striking

curative effects were apparent. The cases did not develop pneumococcus blood infection, and it seems probable that the serum may be able to prevent a general infection from taking place from the diseased lung, even though it may fail to influence the local process. It has also been shown that these injections of antipneumococcic serum are practically harmless. In pneumococcus septicæmia no marked results have been seen. The majority who received the injections, as well as those not receiving them, died. Large intravenous injections of 50 c.c. of a polyvalent serum might be of value.

Vaccines.—The use of injections of dead pneumococci in pneumonia and other acute pneumococcic inflammations has not been followed by appreciable beneficial results in those cases which have come under our observation. In subacute cases the results appear to be more favorable. With better understanding of the proper dosage better results may become possible.

CHAPTER XXVIII.

MENINGOCOCCUS OR MICROCOCCUS (INTRACELLULARIS) MENINGITIDIS, AND THE RELATION OF IT AND OF OTHER BACTERIA TO MENINGITIS.

In the description of the diplococcus of pneumonia reference was made to this organism as the most frequent cause of isolated cases of meningitis, especially when it complicated pneumonia. In 1887 Weichselbaum[1] discovered another micrococcus in the exudate of cerebrospinal meningitis in six cases, two of which were not complicated by pneumonia. He obtained it in pure cultures, studied its characteristics, and showed that this organism was clearly distinguishable from the pneumococcus and especially by its usual presence in the interior pus-cells, on which account he called it *Diplococcus intracellularis meningitidis*. In 1895 Jaeger and Schuerer drew especial attention to the etiological relationship of the organism to the epidemic form of cerebrospinal meningitis. They also believed it to be very probable that in most cases of primary meningitis it is from the mucous membrane of the nasal cavities and the sinuses opening out from them that both the diplococcus of pneumonia and the micrococcus intracellularis find their way through the blood or perhaps directly through the lymph channels to the meninges. The former we know to be almost constantly present in the nasal cavities, and the latter we have reason to believe is not infrequently there. The prevalence of epidemics in winter and spring, a time favorable to influenza and pneumonia, also suggests the respiratory tract as the place of the infection and where an increase in virulence takes place. We do not as yet know why meningitis follows in some persons and not in others after infection of the mucous membranes.

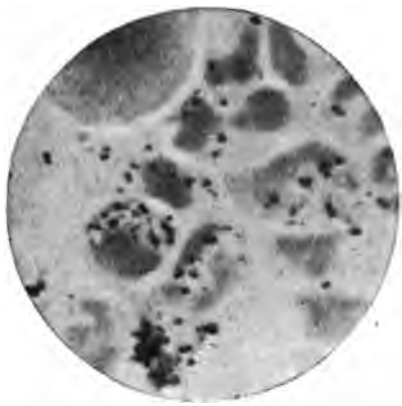
Infected persons as well as things recently soiled by their nasal secretion are dangerous.

Morphology.—This organism occurs as biscuit-shaped micrococci, usually united in pairs, but also in groups of four and in small masses; sometimes solitary and small degenerated forms are found. It has no well-defined capsule. Cultures resemble strongly those of gonococci (see Fig. 127). In cultures more than twenty-four hours old larger and smaller forms occur and some which stain poorly. These are involution forms. In the exudation, like the gonococcus, to which it bears a close resemblance in form and arrangement, it is distinguished by its presence, as a rule, within the polynuclear leukocytes. It never appears within the nucleus and rarely within other cells (Fig. 126).

[1] Weichselbaum. Fortschr. d. Med., 1887, p. 573.

Staining.—It *stains* with all the ordinary aniline colors, but best with Loeffler's methylene blue. It is readily decolorized by Gram's solution. Some organisms in many cultures are more resistant than others, but none are Gram-positive. Elser and Huntoon[1] carefully tested this matter and never found a Gram-positive stain in 200 different cultures. We have had a similar experience in a smaller number. It is almost certain that the positive cocci which have been described by Jaeger and others as meningococci are really contaminating organisms. Stained with Loeffler's alkaline solution of methylene blue the cocci frequently show a central metachromatic granule. The cells have no definite capsule.

FIG. 126



Diplococcus intracellularis meningitidis in pus cells. × 1100 diameters.

Biology.—It grows between 25° and 40° C., best at about 37.5° C., and its development is usually scanty on the surface of nutrient agar, but sometimes a few colonies grow quite vigorously. Now and then cultures grow at 23° C. or slightly less. It grows scarcely at all in bouillon, and scantily in bouillon plus one-third blood-serum. It develops comparatively well on Loeffler's blood-serum medium as used for diphtheria cultures, and on blood-serum or ascitic fluid agar. The addition of 1 per cent. glucose favors growth.

Of the sugars the meningococcus ferments dextrose and maltose only, and even these not sufficiently to coagulate the serum media.

When grown for some time, a tolerably good growth develops at the end of forty-eight hours in the incubator on nutrient agar or glucose agar. This appears as a flat layer of colonies, about one-eighth of an inch in diameter, grayish-white in color, finely granular, rather viscid, and non-confluent unless very close together. On Loeffler's blood serum the growth forms round, whitish, shining, viscid-looking colonies, with smooth and sharply-defined outlines; these may attain diameters of one-eighth to one-sixteenth of an inch in twenty-four hours. The colonies tend to become confluent and do not liquefy the serum. From the spinal fluid in acute cases, where the organisms are apt to be more abundant, a great many minute colonies may develop instead of a few larger ones. On agar plates the deep-lying colonies are almost invisible to the naked eye; somewhat magnified they appear finely granular, with a dentated border. On the surface they are larger, appearing as pale disks, almost transparent at the edges, but more compact toward the centres, which are yellowish-gray in color. On blood agar or serum agar the growth is much more luxuriant than on plain agar and larger than the gonococcus. Not infrequently no growth is obtained when the cerebrospinal fluid containing the diplococci is placed on plain agar, and in rare instances no growth appears when serum agar is used. Cultivated in artificial media, while it often lives for weeks, it may die within four days, and requires, therefore, to be transplanted to fresh material at short intervals—at least every two days.

[1] The Jour. of Med. Research, Vol. xx., No. 4, page 390.

Resistance.—It is readily killed by heat, disinfectants, sunlight, and drying. A few cocci may remain alive for 1 to 3 days in the dried state. To maintain cultures it is necessary to replant recently isolated cultures daily, but after several weeks once a month will suffice. Some cultures are very difficult to keep alive.

Pathogenesis.—This organism does not show marked pathogenic power for adult animals. It is most pathogenic for mice and guinea-pigs, less so for rabbits and dogs. Subcutaneous injections in animals when large cause death.

When mice are inoculated into the pleural or peritoneal cavities they usually fall sick and die within thirty-six to forty-eight hours, showing slight fibrinopurulent exudation.

Certain experiments made by Weichselbaum on dogs, though not entirely successful, are interesting as showing the similarity of the disease produced in them artificially with meningitis as occurring in man. The three dogs, trephined and inoculated subdurally with 0.5 to 2 c.c. of a fresh culture, all died: No. 1 within twelve hours, No. 2 in three days, and No. 3 in twelve days. In Nos. 1 and 2 there were found hyperæmia of the meninges, with inflammatory softening of the brain at the point of inoculation, which on nearer inspection proved to be a true encephalitic process. In dog No. 2, in which the disease was of longer duration, these changes were the most pronounced. Numerous diplococci were observed in the sections removed, for the most part free, but some few within the pus cells. In dog No. 3, in which the disease lasted twelve days, a thick, reddish, purulent liquid was found between the dura mater and the brain at the point of the inoculation; in the brain itself an abscess had formed, about the size of a hazel-nut, filled with tough, yellow pus, while the abscess walls consisted of softened brain substance infiltrated with numerous hemorrhagic deposits. The ventricles on that side contained a cloudy, reddish fluid, with flocks of pus; but no diplococci could be demonstrated in the blood or exudations. In our experience injection of a recent culture into the spinal canal of very young puppies is regularly followed by the results noted by Weichselbaum. Such effects are not observed in older dogs. In monkeys Flexner,[1] von Lingelsheim, and McDonald have been able to produce rather characteristic symptoms and lesions by intraspinal injections. Inoculated in other ways, the usual type of infection was not produced.

Presence in the Nasal Cavity of the Sick and Those in Contact with Them.—In 1 of his 6 cases Weichselbaum succeeded in obtaining diplococci from the nasal secretion. In 1901 Albrecht and Ghon demonstrated them in healthy individuals. Scheurer, in his 18 cases, found the diplococci in the nasal secretions of all of them during life. In 50 healthy individuals examined they were found in the nasal secretions of only two, one being a man suffering at the time from a severe cold. This man, it is interesting to note, had been employed in a

[1] Flexner. Jour. Exp. Med., 1907, ix., page 142.

room which had just previously been occupied by a patient with cerebrospinal meningitis. Lately, there has been a tendency to throw doubt on these findings, but from our experience in the 1906 epidemic in New York, one can state that the meningococci are usually present in great numbers in the nose and naso-pharynx in most cases of meningitis during the first twelve days of illness. After the fourteenth day they cannot usually be found. In one case Goodwin of our laboratory obtained them on the sixty-seventh day. She also found them in five persons out of sixty tested who had been in close contact with the sick, and in two of fifty medical students.

Pathogénicity for Man.—The most marked lesions occur at the base of the brain. The cord is always affected. This is not true to the same extent in other bacterial infections. In some epidemics the course of the disease is very rapid. The mortality varies from between 50 and 80 per cent.

Complicating Infections.—Occasionally we find secondary to the cerebrospinal meningitis, and due to the micrococcus, inflammations of nasal cavities and their accessory sinuses, also catarrhal inflammations of the middle ear, acute bronchitis, and pneumonia. The absolute determination of the identity of the micrococcus found in these conditions has not been established, so that the above complications can only be considered as probably due to this organism.

Except in cases of meningitis the micrococcus has been absolutely identified only in cases of rhinitis. Several observers believe they have found it in the diseases mentioned above as occasionally complicating meningitis.

Meningococci in the Blood.—Elser in forty cases examined during the early days of the disease found them in ten.

Agglutination Characteristics.—A considerable percentage of cultures of meningococci are relatively inagglutinable. Strains that are agglutinable respond to the agglutinins developed in an animal immunized with a true strain. Careful absorption tests are capable in almost, if not all, instances of separating true meningococci from other Gram-negative organisms. The serum reaction is rarely used in diagnosis. The microscopic test is so much more definite.

Serum Treatment.—It is difficult to apportion the credit for the production of the first protective serum. Bonhoff and Lepriere produced in animals a serum which showed definite protection. The world-wide epidemic beginning in 1904 stimulated a number of laboratories to produce sera in horses with the idea of treating human cases.

Thus Kolle[1] and Wasserman, Jochmann, Flexner, and ourselves immunized horses. The usual method was to begin with cultures recently obtained from human cases and grow them on ascitic agar or plain nutrient agar in tubes. The growth was scraped off, added to physiological salt solution, and heated to 55 ° to 60° for one hour. Living cultures were often substituted later.

The original injections were quite small, being only one or two

[1] Deutsche Med. Woch., 1906, xxxii., No. 16.

moderate-sized platinum loopfuls. Each succeeding injection was doubled in size each time, until the maximum dose of the growth on two Petri dishes was given, when the size of the injection was not changed. The injections were given about every eight days. Horses give the best serum after eight months to one year. Kolle and Wasserman injected one horse with the watery extract of recent cultures. They also used both the intravenous and subcutaneous methods.

THE THERAPEUTIC USE OF SERUM.—In 1905 there was inaugurated in Hartford, the use of subcutaneous injections of diphtheria antitoxic serum in meningitis. This influenced us to prepare and try the subcutaneous injection of an antimeningococcus serum. The results reported by the physicians in some twenty cases did not seem to establish that any beneficial effects were obtained, so no further serum was issued. Later Kolle[1] and Wasserman reported somewhat favorable results in a number of cases from the subcutaneous injection of a serum prepared by them. Meanwhile a serum prepared by Jochmann was employed by the intraspinal method in a series of cases. This method soon supplanted the subcutaneous injections.

The first successful use of an immune serum in cases of human cerebrospinal meningitis, by the intraspinal method, should, therefore, so far as we know, be credited to Jochmann,[2] and the physicians who used his serum in the winter of 1905 and 1906. He reported a series of cases treated by the intraspinal method before the Congress for Internal Medicine held in Munich in April, 1906, and published his paper on May 17th, 1906.

The serum was prepared by injecting horses with increasing doses of meningococcus killed at about 58° C. The doses were given every eight days, beginning with a loopful and increasing until the growth on the surface of ascitic agar covering two Petri dishes was used. After this dose was reached living cultures were given.

CHARACTERISTICS OF SERUM.—The serum was shown to possess both bactericidal and opsonic power. He reported forty cases of cerebrospinal meningitis which had been treated, but gave details concerning 17 patients which all occurred in one hospital and were treated by Kromer. Five of these patients died and twelve recovered, a mortality of 29 per cent. He directed that after lumbar puncture, 20 to 50 c.c. of fluid should be removed and then 20 c.c. of immune serum injected. These injections should be repeated once or twice if the fever did not abate or returned. He noticed in general a bettering of the headache, stiffness of neck, and mental condition. Jochmann showed that in animals colored fluids injected into the spinal canal in the lumbar region passed the full length of the canal.

Although the serum prepared in different laboratories in Europe was regularly used after Jochmann's report, it did not receive much attention in the country until Flexner, through his important experiments on infected monkeys, which demonstrated the value of the intra-

[1] *Ibid.*, 1907, xxxiii., p. 1585.
[2] Deutsche med. Wochenschrift, Vol. xxii., No. 20, page 788.

spinal injections of the serum, aroused medical interest and paved the way for him to try out the serum on a large scale. All cases treated by him were subjected to most careful bacterial tests and clinical observation. Eighteen months later, Flexner and Jobling published their report which fully corroborated the earlier results of Jochmann. The serum prepared at the Rockefeller Institute for Medical Research has been sent to many places, both in this country and in Europe. The results obtained have been of the utmost value in arriving at the value of the intraspinal treatment. The following is abstracted from their latest report.[1]

There were 712 cases of the disease in which the bacteriologic diagnosis was made and the serum treatment used. In the first table the cases are subdivided according to certain age periods, and in the second the total cases of each age period are further subdivided according as the serum was injected in the three arbitrarily chosen periods of duration of the disease.

TABLE I.

CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS TREATED WITH THE ANTIMENINGITIS SERUM.

CASES ANALYZED ACCORDING TO AGE GROUPS.

Age, years	Total No. cases	Recovered	Died	% Mortality
1–2	104	60	44	42.3
2–5	112	82	30	26.7
5–10	113	95	18	15.9
10–15	101	73	28	27.7
15–20	107	72	35	32.7
20+	175	106	69	39.4
Total, all ages	712	488	224	31.4

The highest mortality is shown to have occurred in the first two years of life. But, contrary to the rule, under the older forms of treatment in which the mortality was 90 per cent. or over, in this series it was 42.3 per cent. The average mortality in all the age periods was 31.4 per cent.

TABLE II.

CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS TREATED WITH THE ANTIMENINGITIS SERUM.

CASES ANALYZED ACCORDING TO DAY OF INJECTION.

	Period of Injection—Day.								
	1st–3rd			4th–7th			Later than 7th		
Age, years	Rec.	Died	%	Rec.	Died	%	Rec.	Died	%
1–2	16	1	5.8	22	10	31.2	22	33	60.
2–5	24	6	20.	40	12	23.	18	12	40.
5–10	43	8	15.6	35	6	14.6	17	4	19.
10–15	36	8	19.	23	9	28.1	14	11	40.
15–20	25	17	40.4	25	8	24.2	22	10	31.2
20+	36	21	36.8	34	24	41.3	36	24	40.
Totals	180	61	25.3	179	69	27.8	129	94	42.1

[1] Journal of the American Med. Assn., Oct. 30, 1909, Vol. liii., p. 1443.

"Table II is instructive in bringing out the importance of early injections of the serum. The results in the first two years of life are especially noteworthy. The extraordinary figures given under the first period of injection, namely, in the first three days of the disease, can hardly be maintained. But the influence of period of injection is shown by the rapid rise in mortality in the subsequent two periods. The rule of the effects of early injection is preserved in the age periods up to the period of from 15 to 20 years, when it disappears. The discrepancy occurring in the two highest age periods cannot be wholly accounted for at present. The explanations which suggest themselves are that among older individuals there tends to be a large number of very severe, rapidly fatal or fulminating cases of the disease, or that older persons are less subject to the beneficial action of the serum. As regards the actual proposition, it may be stated that adults not infrequently respond promptly to the serum injections by abrupt termination of the disease or ameliorated symptoms and pathologic conditions.

"The total figures do not, however, fail to indicate that the early injections are more effective than the later ones, as is shown by the percentage mortality in the first-to-third-day period of 25.3, in the fourth-to-seventh-day period of 27.8, and the period later than the seventh day of 42.1."

Our own experience and our conversations with a number of physicians both here and in Europe convince us that the intraspinal injections of the serum are of great advantage in the majority of cases and should always be given. We would not advise waiting for a bacteriological examination of the spinal fluid before giving the first injection of the serum. Later injections should be guided by the result of the examination if that has been possible. We feel that the results tabulated by Flexner are a little too favorable because the necessity of having a bacterial examination tended to eliminate the most rapidly fatal cases. It is also true that the comparison between the treated cases in which meningococci were found and the untreated cases in which no such bacterial tests were made gives a too favorable contrast. It is well known that a considerable number of cases due to pneumococci, streptococci, and tubercle bacilli are diagnosed as cerebrospinal meningitis. These are almost invariably fatal. These facts, however, do not lessen our conviction of the great usefulness of the serum, but simply reduce somewhat the degree to which we believe the mortality has been reduced.

Bacteriological Diagnosis.—By means of lumbar puncture, fluid can readily be obtained from the spinal canal without danger. The skin must be thoroughly cleansed and the needle aseptic. The fluid should be placed in a sterile conical glass to settle. The sediment should be used to make smears to examine (1) for pus cells, (2) for tubercle bacilli, and (3) for other organisms. By Gram's stain we are able to separate the three Gram-positive organisms most frequently met with in meningitis (pneumococcus, streptococcus, and

staphylococcus) from the others. Of importance also is the point that the micrococcus intracellularis is usually inside the leukocytes in the form of diplococci of varying size, of coffee-bean shape, or of tetracocci, while the pneumococcus is frequently outside the cells and is usually spherical or lancet-shaped and frequently occurs in short chains. Sometimes the bacteria are present in very small numbers, and then many smears must be looked through before a probable diagnosis can be made. In all cases absolute certainty can only be obtained through cultures. Here plain nutrient agar, serum agar, and blood-agar plates should be made, and, if desired. tubes also. When considerable quantities are inoculated upon these media and meningococci are present, as a rule, a greater or less number of colonies having the characteristics already described will develop. The value, clinically, of the examination is that about 50 per cent. of the cases due to this coccus recover, while almost all of those due to the pneumococcus and streptococcus die.

In many cases there are very few diplococci present in the spinal fluid, so that a failure to find them in a microscopic examination should not be taken to prove that the disease was not due to this organism. In two cases we could find no diplococci in the fluid withdrawn on the first day of the disease, but found them on the second day. In 210 cases examined Elser and Huntoon[1] made a positive microscopic or cultural diagnosis in 92.4 per cent. Of 171 examined microscopically, 141 were positive. During the first week of the disease of 120 examined, the films were positive in 100. Of 177 examined by cultures, 152 were positive.

They believe that if cultures could have been made immediately upon the withdrawal of the fluid better results could have been attained. The number of organisms tend to diminish as the disease advances. In a total of 152 consecutive isolations of Gram-negative cocci from the spinal canal they all proved on the most rigid tests to be meningococci. A finding of Gram-negative organisms in leukocytes is sufficient for a diagnosis. Some observers have considered that gonococci occasionally excited meningitis. This is possible, but it seems more likely that it was a mistake in identification. A few Gram-negative cocci have been reported by different observers, but it seems likely that there was contamination. These cocci were usually free in the fluid. For cultures a considerable amount of fluid must be used, for we have found, as described by Councilman and others, that there may be very few living diplococci even in 1 c.c. of fluid.

To obtain the fluid the patient should lie on the right side with the knees drawn up and the left shoulder depressed. The skin of the patient's back, the hands of the operator, and the large antitoxin syringe should be sterile. The needle should be 4 cm. in length, with a diameter of 1 mm. for children, and longer for adults.

The puncture is generally made between the third and fourth

[1] Elser and Huntoon. *Ibid.*, p. 400

lumbar vertebræ. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is entered in the median line or a little to the right of it, and on a level with the thumb-nail, and directed slightly upward and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 cm. in adults the needle enters the subarachnoid space, and on withdrawing the obturator the fluid flows out in drops or in a stream. If the needle meets a bony obstruction withdraw and thrust again rather than make lateral movements. Any blood obscures the microscopic examination. The fluid is allowed to drop into absolutely sterile test-tubes or vials with sterile stoppers. From 5 to 15 c.c. should be withdrawn. No ill effects have been observed from the operations. On the contrary, the relief of pressure frequently produces beneficial results.

Differential Diagnosis from Gonococci.—As a rule, the portion of the body from which the organisms are obtained reveals their source. When this is insufficient careful culture and agglutination tests are required.

Other Organisms Exciting Meningitis.—1. The *pneumococcus.* This diplococcus is one of the most frequent exciters of meningitis, both as a primary and a secondary infection.

2. The *Streptococcus pyogenes* and the *Staphylococcus pyogenes.* Meningitis due to these organisms is almost always secondary to some other infection, such as otitis, tonsillitis, erysipelas, endocarditis, suppurating wounds of scalp and skull, etc.

3. The *Bacillus influenzæ.* Numerous reports have been published of the presence of influenza bacilli in the meningeal exudate. Those that are reliable state in almost every instance that the meningitis is secondary to infection of the lungs, bronchi, the nasal cavities or their accessory sinuses.

4. The *colon bacillus*, the *typhoid bacillus*, that of *bubonic plague* and of *glanders*, all may cause a complicating purulent meningitis.

5. In isolated cases of meningitis complicating otitis media and other infections, other bacteria, such as the *Micrococcus tetragenus*, the *Bacillus pyocyaneus*, the *gonococcus*, etc., may be found.

6. The *tubercle bacillus.* This is a very frequent cause.

MICROCOCCUS CATARRHALIS (R. PFEIFFER).

Micrococci somewhat resembling meningococci are found in the mucous membranes of the respiratory tract. At times they excite catarrhal inflammation of the mucous membranes and pneumonia. These are at present included under the designation of *Micrococcus catarrhalis.*

Microscopic Appearance.—They usually occur in pairs, sometimes in fours; never in chains. The cocci are coffee-bean in shape and slightly larger than the gonococcus, and are negative to Gram's stain.

The micrococci are not motile and produce no spores.

Cultivation.—They grow between 20° and 40° C., best at 37° C. and less rapidly at somewhat lower temperatures, developing on ordinary nutrient agar as grayish-white or yellowish-white, circular colonies of the size of meningococci. The borders of the colonies are irregular and abrupt as though

gouged out. They have a mortar-like consistency. On serum-agar media the growth is more luxuriant. Gelatin is not liquefied. Bouillon is clouded, often with the development of a pellicle. Milk is not coagulated, but dextrose serum media may be. Gas is not produced.

Location of Organisms.—In the secretion of normal mucous membranes they are occasionally present. In certain diseased conditions of the mucous membranes they may be abundant.

Pathogenic Effects in Animals.—For white mice, guinea-pigs, and rabbits, some cultures are as pathogenic as meningococci, while others are less so.

Differential Points Separating them from the Meningococci.—These organisms have undoubtedly been at times confused. Some assert that the meningococci grow only above 25° C. Many cord cultures of meningococci grow below this point. Some assert that the meningococci will not grow on 5 per cent. glycerin agar. Many undoubted cultures do. Careful agglutinin absorption tests are of great differential value, but can only be carried out safely by one accustomed to them. The meningococci tested by us have removed all the agglutinins acting upon meningococci from a specific meningococcus serum while the allied organisms have removed only about sixty per cent. of them. The probability is that the organisms described by different writers as *Micrococcus catarrhalis* were not all the same variety, and some of them were meningococci.

Vaccine Therapy.—Good reports have been made of the results of injecting the dead organisms in a number of infections due to this micrococcus.

OTHER GRAM-NEGATIVE COCCI RESEMBLING MENINGOCOCCI.

Pseudomeningococcus.—This organism cannot be differentrated from meningococci except by serum reactions.

Micrococcus pharyngis siccus (Von Lingelsheim[1]).—Diplococcus mucosus.—Chromogenic Gram-negative cocci.

[1] Klin. Jahrb., 1906, xv., H. 2.

CHAPTER XXIX.

THE GONOCOCCUS OR MICROCOCCUS GONORRHŒÆ—THE DUCREY BACILLUS OF SOFT CHANCRE—MICROCOCCUS MELITENSIS.

THE period at which gonorrhœa began to inflict man is unknown. The earliest records make mention of it. Wherever civilized man has penetrated gonorrhœa is prevalent among the people. Except for a period after the fifteenth century it was generally recognized as a communicable disease and laws were made to control its spread. The differentiation between the lighter forms of gonorrhœa and some other inflammations of the mucous membranes was, however, almost impossible until the discovery of the specific microörganism by Neisser, in 1879.

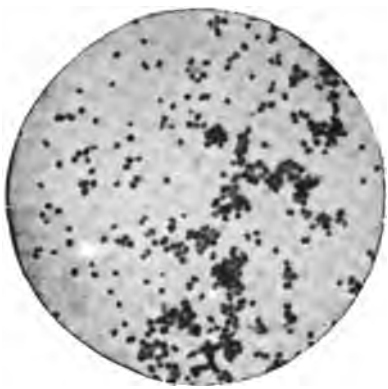
The organism was first observed in gonorrhœal discharges, and, described by him under the name of "gonococcus;" but though several attempted to discover a medium upon which it might be cultivated, it was reserved for Bumm, in 1885, to obtain it in pure culture upon coagulated human-blood serum, and then after cultivating it for many generations to prove its infective virulence by inoculation into man. The researches of Neisser and Bumm established beyond doubt that this organism is the specific cause of gonorrhœa in man. Gonorrhœa is in almost all cases among adults transmitted through sexual intercourse. Gonorrhœal ophthalmia is a frequent accidental infection at birth and vaginitis in the young child is frequently produced by the carelessness of the nurse or mother carrying infection.

Microscopic Appearance.—Micrococci, occurring mostly in the form of diplococci. The bodies of the diplococci are elongated, and, as shown in stained preparations, have an unstained division or interspace between two flattened surfaces facing one another, which give them their characteristic "coffee-bean" or "kidney" shape. The older cocci lengthen, then become constricted in their middle portion, and finally divide, making new pairs (Fig. 127). The diameter of an associated pair of cells varies according to their stage of development from 0.8μ to 1.6μ in the long diameter—average about 1.25μ—by 0.6μ to 0.8μ in the cross diameter.

Extracellular and Intracellular Position of Gonococci.—In gonorrhœa, during the earliest stages before the discharge becomes purulent, the gonococci are found mostly free in the serum or plastered upon the epithelium cells, but later almost entirely in small, irregular groups in or upon the pus cells, and always extranuclear. With the disappearance of the pus formation more free gonococci appear. Discharge expressed from the urethra usually contains more free organisms than the natural flow. Gonococci are sometimes irregular in shape or

granular in appearance, involution forms, found particularly in older cultures and in chronic urethritis of long standing. Single pus cells sometimes contain as many as one hundred gonococci and seem to be almost bursting and yet show but slight signs of injury. These diplococci are also found in or upon desquamated epithelial cells. There is still discussion as to whether the gonococci actively invade the pus cells or only are taken up by them. There is no evidence that the gonococci are destroyed by the pus cells (Fig. 128).

FIG. 127



Smear from pure culture of gonococcus on agar. × 1100 diameters. (Heiman.)

FIG. 128

Gonococcus in pus cells. × 1100 diameters

Staining.—The gonococcus *stains* readily with the basic aniline colors. Loeffler's solution of methylene blue is one of the best staining agents for demonstrating its presence in pus, for, while staining the gonococci deeply, it leaves the cell protoplasm but faintly stained. Fuchsin is apt to overstain the cell substance. Beautiful double-stained preparations may be made from gonorrhœal pus by treating cover-glass smears with methylene blue and eosin. Numerous methods for double staining have been employed, with the object of making a few gonococci more conspicuous. None of them have any specific characteristics such as the Gram stain. It is now established that gonococci from fresh cultures and from recent gonorrhœal infections are, when properly treated by Gram's method, quickly and surely robbed of their color and take on the contrast stains. The removal of the stain from gonococci in old flakes and threads from chronic cases is not so certain. This difference is mostly due to the fact that equally uniform specimens cannot be prepared. The decolorized gonococci are stained by dipping the films for a few seconds into a 1 : 10 dilution of carbol-fuchsin or a solution of bismarck brown. This staining should be for as short a time as suffices to stain the decolorized organisms. This method of staining cannot be depended upon alone absolutely to distinguish the gonococcus from all other diplococci found in the urethra and vulvo-vaginal

tract, for, especially in the female, other diplococci are occasionally found which are also not stained by Gram's method. It serves, however, to distinguish this micrococcus from the common pyogenic cocci, which retain their color when treated in the same way, and in the male urethra it is practically certain, as no organism has been found in that location which in morphology and staining is identical with the gonococcus. It is certainly the most distinctive characteristic of the staining properties of the gonococcus, and it is a test that should never be neglected in differentiating this organism from others which are morphologically similar.

Biology.—Grows best at blood temperature; the limits being roughly 25° and 40° C. It is a facultative anaërobe. It is not motile and produces no spores.

Culture Media.—The gonococcus requires for its best growth the addition to nutrient agar of a small percentage of blood serum or some equivalent. The following media have proven of value:

1. Human blood from the sterilized finger streaked on common nutrient agar.

2. Human ascitic, pleuritic, or cystic fluid, 1 part added to and mixed with 2 parts melted 5 per cent. glycerin nutrient, 1.5 per cent. agar having a temperature of 55° to 60° C. The whole after mixing being poured into a Petri dish or cooled slanted in a tube. The same proportions of nutrient broth and ascitic fluid make a suitable fluid medium. One per cent. glucose may be added.

3. Swine serum nutrose media. Wassermann strongly recommends this mixture. In our hands it has given good results.

4. Nutrient or 5 per cent. glycerin agar. When considerable pus is streaked on simple agar media a good growth of gonococci is usually obtained. After continued cultivation gonococci cultures frequently grow on media containing no serum. Some strains grow on ordinary glycerin or glucose nutrient agar and even on plain nutrient agar from the start.

Viability.—Cultures frequently die in forty-eight to seventy-two hours when kept at room temperature. In the ice-box they may live for several weeks. They frequently live for one week in the thermostat at 36° C. on plain nutrient agar.

Appearance of Colonies.—A delicate growth is characteristic. At the end of twenty-four hours there will have developed translucent, very finely granular colonies, with scalloped margin. The margin is sometimes scarcely to be differentiated from the culture medium. In color they are grayish-white, with a tinge of yellow. The texture is finely granular at the periphery, presenting punctated spots of higher refraction in and around the centre of yellowish color (Fig. 129).

Surface Streak Culture.—Translucent grayish-white growth, with rather thick edges.

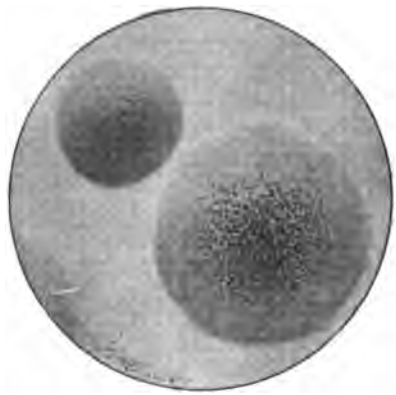
Resistance.—The gonococcus has but little resistant power against

outside influences. It is killed by weak disinfecting solutions and by desiccation in thin layers. In comparatively thick layers, however, as when gonorrhœal pus is smeared on linen, it has lived for forty-nine days, and dried on glass for twenty-nine days (Heiman). It is killed at a temperature of 45° C. in six hours and of 60° in about thirty minutes.

Occurrence of Gonococci.—Outside of the human body or material carried from it gonococci have not been found.

Toxins.—In the gonococcus cells substances are present which are toxic after heating and contact with alcohol. Injected in considerable amounts into rabbits, they cause infiltration and often necrosis. Applied to the urethral mucous membrane there is produced an inflammation of short duration. In gonorrhœa the secretion is believed to be due to these intracellular toxins. Repeated injections give only slight immunity. The filtrate of recent gonococcus cultures contains little toxin.

FIG. 129



Colonies of gonococci on pleuritic fluid agar. (Heiman.)

Pathogenesis.—Non-transmissible to all animals. Both the living and dead gonococci contain toxic substances which cause death or injury when injected in large quantities.

The etiological relation of the gonococcus to human gonorrhœa has been demonstrated beyond question by the infection of a number of healthy men with the disease by the inoculation of pure cultures of the microörganism.

Disease Conditions Excited by Gonococci.—Affections due to this organism are usually restricted to the mucous membranes of the urethra, prostate, neck of bladder, cervix uteri, vagina, and conjunctiva. The conjunctival, vaginal, and rectal mucous membranes are much more sensitive in early childhood than in later life. The usual course of the inflammation is as follows: The gonococci first increase upon the mucous membranes which show congestion, infiltration with serous exudate and accumulation of leukocytes. The cocci then penetrate the epithelial layer down to the submucous connective tissue. Recovery or a prolonged chronic inflammation may then persist. The original infection of the urethra or vagina and cervix may remain localized or spread to adjacent parts or through blood and lymph be carried to all parts of the body. Gonococci thus cause many cases of endometritis, metritis, salpingitis, oöphoritis, peritonitis, prostitis, cystitis, epididymitis, and arthritis. Abscesses of considerable size, periostitis, and otitis are occasionally due to the gonococcus.

Endocarditis and Septicæmia.—Cases of gonococcus endocarditis and septicæmia are not infrequent. Gonococcus septicæmia may occur in connection with other localizations or alone. Nearly every year one or two of these cases are met with in every general hospital. In a considerable number of cases where gonococci are obtained from the blood the patients recover. The fever is sometimes typhoid-like in character.

Complications.—General infections with gonococci are often followed or accompanied by neuralgic affections, muscle atrophies, and neuritis. Urticaria occasionally occurs.

Immunity.—Immunity in man after recovery from infection seems to be only slight in amount and for a short period if present at all. It is known that the urethra in man or cervix uteri in woman may contain gonococci which lie dormant and may be innocuous in that person for years, but which may at any time excite an acute gonorrhœa in another individual or, under stimulating conditions, in the one carrying the infections. Animals may, however, be immunized, and their blood is both bactericidal and slightly antitoxic.

Therapeutic Use of Serum and Vaccine.—The use of sera in acute gonorrhœal joint inflammation has given in a considerable percentage of cases good results and seems to be worth trying. It seems to be useless in acute gonorrhœa of the mucous membranes. Vaccines (heated cultures) have also been used with apparently real benefit in joint inflammations and even in very localized chronic infections of the urethra, bladder, and elsewhere. They have also been used in acute vaginitis in young children. In our cases the symptoms abated sooner than we expected, but the gonococci persisted. The dose is from twenty to a thousand millions given every three to seven days. The benefit of serum and vaccine in septicæmia is doubtful.

Agglutination.—Torrey has shown that gonococci resemble pneumococci in that there are a number of different strains which have different specific and but little common agglutinins. The agglutination test is of no practical value in diagnosis.

Duration of Infections and of Contagious Period.—There is no limit to the time during which a man or woman may remain infected with gonococci and infect others. We have had one case under observation where twenty years had elapsed since exposure to infection, and yet the gonococci were still abundant. It is now well established that most of the inflammations of the female genital tract are due to gonococci, and the majority of such infections are produced in innocent women by their husbands who are suffering from latent gonorrhœa.

Bacteriological Diagnosis of Gonorrhœa.—In view of the fact that several non-gonorrhœal forms of urethritis occasionally exist, and also that micrococci morphologically similar to the gonococcus Neisser are at times found in the normal vulvo-vaginal tract of adults it becomes a matter of importance to be able to detect gonococci when present, and to differentiate these from the non-specific or-

ganisms. Besides this, the gonococci which occur in old cultures and in chronic urethritis of long standing sometimes take on a very diversified appearance. From a medicolegal and social standpoint, therefore, the differential diagnosis of the gonococcus has in certain cases a very practical significance.

There are two methods of differential diagnosis now available—the microscopic and the cultural. Animal inoculations are of no value, as animals are not susceptible, and, of course, human inoculations are generally impossible. In the microscopic diagnosis it should be born in mind that after the acute serous stage has passed, the specific gonococci in *carefully made* preparations are always found largely within the pus cells. Diplococci morphologically similar to gonococci occuring in other portions of the field and outside of the pus cells should not be considered specific by the test only. It should also be remembered that the gonococci are decolorized by Gram's method, while other similar micrococci which occur in the urethra are, as a rule at least, not so decolorized. Organisms having these characteristics can for all practical purposes be considered as certainly gonococci if obtained from the urethra. From the vulvovaginal tract the certainty is not so great, since other diplococci are occasionally found in gonorrhœal pus from this area, and very rarely, also, from the urethra, which stain as gonococci; here cultures should also be made.

Cover-glass preparations from subacute or chronic cases should be examined, if possible, with a microscope provided with a mechanical stage, and films should always be stained by both Loeffler's methylene-blue solution and by Gram's method, and the examination repeated on three consecutive days. Should these specimens prove negative, to exclude any possible doubt in the matter, cultures should then be made, if a thoroughly competent bacteriologist is available, on human ascitic fluid or serum agar, poured in dishes; also, if with negative results, on three consecutive days. Heiman, who has paid much attention to gonococcus examinations, obtains his material by the following method: in chronic urethritis he allows the patient to void his urine either immediately into two sterilized centrifugal tubes or first into two sterile bottles. The first tube will contain threads of the anterior urethra; the second tube will be likely to contain secretion from the posterior urethra and from the prostate gland if, while urinating, the patient's prostate be pressed upon with the finger. Tubes containing such urine are placed in the centrifuge and whirled for three minutes at twelve hundred or more revolutions per minute; the threads are thrown down. The centrifuged sediment will be found to contain most of the bacteria present, epithelial cells, and, at times, spermatozoa. Normal urine on being centrifuged at this velocity will be found at times to show a slight turbidity at the bottom of the tube. This will be found, on microscopic examination, to consist of epithelial cells, a few leukocytes, and some bacteria.

The careful examination of gonorrhœal threads stained by Gram's

method is a very tedious affair, as in every instance no less than three cover-glass preparations should be looked over before the absence of the gonococcus is considered probable. It would require many hours upon each and every specimen, especially if the gonococci are present in very small number, before a reliable and conscientious opinion could be rendered. If, after all, a negative opinion is ventured, we still are under the necessity of proving that because the threads which we fished out for the cover-glass examination were free from gonococci the remaining ones were also. For this reason the culture medium is more sensitive for bacteria than is the cover-glass, for we are able to plant each and every thread of the sediment in the centrifugal tube. Results on culture media are only reliable when obtained by thoroughly trained bacteriologists with suitable media and methods. Fürbringer, in his work, mentions the fact that in certain cases the absence of the gonococcus in many examinations of cover-glass preparations is not a positive proof that the gonococcus is not present. The culture methods, of course, presuppose that one has the facilities and knowledge to carry them out successfully, otherwise the microscopic methods are to be used alone.

When the examinations are negative and it is important to be certain, either massage or injections of a solution of silver nitrate may be employed. The latter by causing a temporary irritation with increase of secretion will almost surely causea discharge of gonococci if any infection was present.

In acute cases where the pus is abundant the specimen for examination may be collected, when the patient is before one, by passing a sterilized platinum-wire loop as far up into the urethra as possible and withdrawing some of the secretion.

Occurrence in Cultures from Chronic Urethritis.—Goll examined 1046 cases of chronic urethritis varying in duration between four weeks to six years or more, finding gonococci in 178 cases, the remainder giving negative results. Neisser, out of 143 cases, varying in duration between two months and eight years, found gonococci in 80 cases.

BACTERIA RESEMBLING GONOCOCCI.

Baumm described a number of micrococci which resembled gonococci in form and staining. These assume importance largely because they may be confused with the gonococcus. They occur on the conjunctival and vaginal mucous membranes and cause confusion. One of these microörganisms, the *Micrococcus catarrhalis* (see p. 400), has an importance of its own. When absolute certainty is demanded cultural tests must be applied.

MALTA FEVER.

The Micrococcus Melitensis.—This microörganism was first discovered in the spleen in a case of Malta fever by Bruce in Malta in 1887. The disease is mostly confined to the shores of the Mediterranean, but cases of it have been observed in Porto Rico, China, Japan,

and the Philippines. The disease does not seem to be directly transmitted from person to person.

Clinical Symptoms.—Prodromal symptoms follow an incubation period of five to fourteen days. Headache, sleeplessness, loss of appetite, or vomiting accompany a high fever. The fever lasts for weeks, with intermissions and remissions. The fever periods of one to three weeks may occur from time to time during a period of many months. The spleen and liver are enlarged. Neuralgic pains are severe. The fatal cases appear similar to severe cases of typhoid fever.

Autopsy.—The spleen is large and very soft. The liver is also large and congested. Both organs show parenchymatous degeneration.

Distribution of Micrococci.—These are abundant in the blood and all organs.

Morphology and Biology.—Very small rounded or slightly oval organisms, about 0.30μ in their greatest diameter. It is usually single or in pairs. In old cultures involution almost bacillary forms occur. They are not motile.

Staining.—They stain readily with aniline dyes and are negative to Gram.

Cultivation.—At 37° C. they grow rather feebly on nutrient agar and in broth. The colonies are not usually visible until the third day. They appear as small round disks, slightly raised, with a yellowish tint in the centre. The broth is slightly clouded after four to six days. The culture remains alive for several weeks or months. In gelatin the growth is very slow. Gelatin is not liquefied.

Pathogenesis in Animals.—Monkeys only are infected. They pass through the disease much like man. They can be infected by subcutaneous or mucous inoculation. In Malta it has been found that about half of the goats pass the organisms in fæces and so contaminate their milk. This is believed to be a source of infection. By safeguarding the milk the disease has been largely eliminated.

Therapeutic Results.—Injections of heated cultures have been thought to give good results.

Methods of Diagnosis.—The diagnosis of Malta fever can frequently only be made with the help of bacteriological examination. Malaise, typhoid fever, and sepsis are the three diseases most apt to be confounded with it.

Cultures are made by spreading over the surface of a number of agar plates freshly drawn blood. Frequently no organisms develop. The agglutination test is then required. Many bloods of persons suffering from other infections agglutinate the micrococcus of Malta fever in low dilutions so that 1:500 or over is required for a positive diagnosis.

Animals injected with the coccus produce a serum agglutinating in high dilutions. Under suitable precautions this can be used to identify suspected cultures.

Laboratory Infection.—A number of workers have infected themselves with more or less serious results.

MICROCOCCUS ZYMOGENS.

MacCallum and Hastings[1] observed this micrococcus in a case of acute endocarditis. It has since been found in a few other pathologic processes. It occurs in pairs and short chains. It grows well on agar, ferments lactose and glucose, and slowly liquefies gelatin.

THE BACILLUS OF SOFT CHANCRE.

This bacillus was first specifically described and obtained in pure culture by Ducrey in 1889. An experimental inoculation is followed in one to two days by a small pustule. This soon ruptures and a small round depressed ulcer is left. About this other pustules and ulcers develop which tend to become confluent. The base of the ulcer is covered with a gray exudate and its edges are undermined. There is no induration such as in the syphilitic chancre. The secretion is seropurulent and very infectious.

Morphology.—About 1.5μ long and 0.4μ thick, growing often in chains and in cultures, sometimes twisted together in dense masses.

It *stains* best with carbol-fuchsin, and shows polar staining.

Cultural Characteristics.—The following method of cultivation has given the best results: Two parts agar are liquefied at 50° C. and mixed with one part human, dog, or rabbit blood. The blood from the cut carotid of a rabbit may be allowed to run directly into the agar tube, to which the pus from the ulcerated bubo is then added in proper proportion, and the whole placed in the incubator at 35° C. The pus may be obtained by puncture and aspiration from the unbroken ulcer, or if the ulcer is already open it is first painted with tincture of iodine and covered with collodion or sterile gauze. After twenty-four to forty-eight hours, some pus having collected under the bandage, inoculations are made from it. The bacillus grows well also in uncoagulated rabbit-blood serum or in condensation water of blood agar. In twenty-four to forty-eight hours, on the surface of the media, well-developed, shining, grayish colonies, about 1 mm. in diameter, may be observed. The colonies remain separate, but only become numerous after further transplantation. The best results are obtained when the pus is taken close to the walls of the abscess. Glass smears show isolated bacilli or short parallel chains with distinct polar staining.

After the eleventh generation of the culture, and from all old cultures, on inoculation the characteristic soft chancre is produced in man. Animals in general cannot be infected, but positive results have been obtained with monkeys and cats.

The organisms are especially characteristic in the water of con-

[1] Jour. Exp. Med., 1899, iv., p. 521.

densation from blood agar, the bacilli being thinner and shorter, with rounded ends; sometimes long, wavy chains are found. In rabbit-blood serum at 37° C. a slight clouding of the medium is produced and small flakes are formed, consisting of short bacilli or moderately long, curved chains, showing polar staining.

The bacillus lives several weeks on blood agar at 37° C., but it soon dies in cultures on coagulated serum. All other ordinary culture media so far tried have given negative results, and even with the media described development is difficult and often fails entirely.

The chancre bacillus possesses but little resistance to deleterious outside influences. Hence, the various antiseptic bandages, etc., used in treatment of the affection soon bring about recovery by preventing the spread of inoculation chancre.

CHAPTER XXX.

BACILLUS PYOCYANEUS (BACILLUS OF GREEN AND OF BLUE PUS)—BACILLUS PROTEUS (VULGARIS).

BACILLUS PYOCYANEUS.

THE blue and green coloration which is occasionally found to accompany the purulent discharges from open wounds is usually due to the action of the *Bacillus pyocyaneus*. According to recent investigations, this bacillus appears to be very widely distributed and not infrequently the cause of infection. It was first obtained in pure culture and its significance noted by Gessard.

Morphology.—Slender rods from 0.3μ to 1μ broad and from 2μ to 6μ long; frequently united in pairs or in chains of four to six elements; occasionally growing out into long filaments and twisted spirals. The bacillus is actively motile, a single flagellum being attached to one end. Does not form spores. *Stains* with the ordinary aniline colors; does not stain with Gram's solution.

FIG. 130

Bacillus pyocyaneus (From Kolle and Wassermann.)

Biology.—Aërobic, liquefying, motile bacillus. Capable also of an anaërobic existence, but then produces no pigment. Grows readily on all artificial culture media at the room temperature, though best at 37° C., and gives to some of them a bright green color in the presence of oxygen. In *gelatin-plate* cultures the colonies are rapidly developed, imparting to the medium a fluorescent green color; liquefaction begins at the end of two or three days, and by the fifth day the gelatin is usually all liquefied. The deep colonies, before liquefaction sets in, appear as round, granular masses with scalloped margins, having a yellowish-green color; the surface colonies have a darker green centre, surrounded by a delicate, radiating zone. In *stick cultures in gelatin* liquefaction occurs at first near the surface, in the form of a small funnel, and gradually extends downward; later the liquefied gelatin is separated from the solid part of the medium by a horizontal plane, a greenish-yellow color being imparted to

that portion which is in contact with the air. On *agar* a wrinkled, moist, greenish-white layer is developed, while the surrounding medium is bright green; this subsequently becomes darker in color, changing to blue-green or almost black. In *bouillon* the green color is produced, and the growth appears as a delicate, flocculent sediment. *Milk* is coagulated and assumes a yellowish-green color.

Pigment.—Two pigments are produced by this bacillus—one of a fluorescent green which is common to many bacteria. This is soluble in water but not in chloroform. The other (pyocyanin) of a blue color is soluble in chloroform, and may be obtained from pure solution in long, blue needles. This pigment distinguishes the *Bacillus pyocyaneus* from other fluorescing bacteria.

Ferment.—Besides the ferment causing liquefaction of gelatin there is one which acts on albumin. It resists heat. This ferment called pyocyanase has the power to dissolve bacteria, and it has been stated to have some protective power when injected into animals. It has been used locally in diphtheria in a number of cases. We do not think it has any advantage over the cleansing preparations.

Distribution.—This bacillus is very widely distributed in nature; it is found on the healthy skin of man, in the fæces of many animals, in water contaminated by animal or human material, in purulent discharges, and in serous wound secretions.

Pathogenesis.—Its pathogenic effects on animals have been carefully studied. It is pathogenic for guinea-pigs and rabbits. Subcutaneous or intraperitoneal injections of 1 c.c. or more of a bouillon culture usually cause the death of the animal in from twenty-four to thirty-six hours. Subcutaneous inoculations produce an extensive inflammatory œdema and purulent infiltration of the tissues; a serofibrinous or purulent peritonitis is induced by the introduction of the bacillus into the peritoneal cavity. The bacilli multiply in the body, and may be found in the serous or purulent fluid in the subcutaneous tissues or abdominal cavity, as well as in the blood and various organs. When smaller quantities are injected subcutaneously the animal usually recovers, only a local inflammatory reaction being set up (abscess), and it is subsequently immune against a second inoculation with doses which would prove fatal to an unprotected animal. It is interesting to note that Bouchard, Charrin, and Guignard have shown that in rabbits which have been inoculated with a culture of the bacillus anthracis a fatal result may be prevented by inoculating the same animal soon after with a pure culture of the bacillus pyocyaneus. Loew and Emmerich have shown that the enzymes produced in the pyocyaneus cultures are capable of destroying many forms of bacteria in the test-tube, and have a slight protecting value in the body. The pyocyaneus bacillus produces these effects not only through ferments, but by intracellular toxins.

Our knowledge of the pathogenic importance of the *Bacillus pyocyaneus* in human diseases has been much increased by recent investigations. Its presence in wounds greatly delays the process of re-

pair, and may give rise to a general depression of the vital powers from the absorption of its toxic products. This bacillus has been obtained in pure culture from pus derived from the tympanic cavity in disease of the middle ear, from cases of ophthalmia, and bronchopneumonia. Kruse and Pasquale have found the organism in three cases of idiopathic abscess of the liver, in two of them in immense numbers and in pure culture. Ernst and Schürmayer report the presence of the bacillus pyocyaneous in serous inflammations of the pericardial sac and of the knee-joint. Ehlers gives the history of a disease in two sisters who were attacked simultaneously with fever, albuminuria, and paralysis. It was thought that they would prove to be typhoid fever or meningitis, but on the twelfth day there was an eruption of blisters, from the contents of which the bacillus pyocyaneus was isolated. Krambals refers to seven cases in which a general pyocyaneus infection occurred, and adds an eighth from his own experience. In this the bacillus pyocyaneus was obtained post-mortem from green pus in the pleural cavity, from serum in the pericardial sac, and from the spleen in pure culture. Schimmelbusch states that a physician injected 0.5 c.c. of sterilized (by heat) culture into his forearm. As a result of this injection, after a few hours he had a slight chill, followed by fever, which at the end of twelve hours reached 38.8° C.; an erysipelatous-like swelling of the forearm occurred, and the glands in the axilla were swollen and painful. Wassermann reports an epidemic of septic infection of the newborn, starting in the umbilicus. In all there were eleven deaths. Lartigau found it in well-water, and in great abundance in the intestinal discharges of a number of cases made ill by drinking the water. It has also been found in a certain number of cases of gastroenteritis where no special cause of infection could be noted.

We may therefore conclude from these facts that the *Bacillus pyocyaneus*, although ordinarily but slightly pathogenic for man, may under certain conditions, as in general debility, become a dangerous source of infection. Children would seem to be particularly susceptible.

The differential diagnosis of the pyocyaneus from other fluorescing bacteria is easy enough as long as it retains its pigment-producing property. When an agar culture is agitated with chloroform a blue coloration demonstrates the presence of this bacillus. When the pyocyanin is no longer formed, however, the diagnosis is by no means easy, particularly when the pathogenic properties are also gone.

Immunity.—Animal infection is followed by the production of antitoxic and bactericidal substances. No practical use has been made of this knowledge.

BACILLUS PROTEUS (VULGARIS).

This bacillus, which is one of the most common and widely distributed putrefactive bacteria, was discovered by Hauser (1885) along with other species of proteus in putrefying substances. These

bacteria were formerly included under the name "*Bacterium termo*" by previous observers, who applied this name to any minute motile bacilli found in putrefying infusions.

Morphology.—Bacilli varying greatly in size; most commonly occurring 0.6μ broad and 1.2μ long, but shorter and longer forms may also be seen, even growing out into flexible filaments which are sometimes more or less wavy or twisted like braids of hair.

The bacillus does not form spores, and *stains* readily with fuchsin or gentian violet.

Biology.—An aërobic, facultative anaërobic, liquefying, motile bacillus. Grows rapidly in the usual culture media at the room temperature.

Growth on Gelatin.—The growth upon *gelatin plates* containing 5 per cent. of gelatin is very characteristic. At the end of ten or twelve hours at room temperature small, round depressions in the gelatin are observed, which contain liquefied gelatin and a whitish mass consisting of bacilli in the centre. Under a low-power lens these depressions are seen to be surrounded by a radiating zone composed of two or more layers, outside of which is a zone of a single layer, from which amœba-like processes extend upon the surface of the gelatin. These processes are constantly undergoing changes in their form and position. The young colonies deep down in the gelatin are somewhat more compact, and rounded or humpbacked; later they are covered with soft down; then they form irregular, radiating masses, and simulate the superficial colonies. But it is difficult to describe all the forms which the proteus vulgaris takes on in all the stages of its growth on gelatin plates. When the consistency of the medium is more solid, as in 10 per cent. gelatin the liquefaction and migration of surface colonies are more or less retarded. In *gelatin-stick* cultures the growth is less characteristic—liquefaction takes place rapidly along the line of puncture, and soon the entire contents of the tube are liquefied.

Upon *Nutrient agar* a rapidly spreading, moist, thin, grayish-white layer appears, and migration of the colonies also occurs. *Milk* is coagulated, with the production of acid.

Cultures in media containing albumin or gelatin have a disagreeable, putrefactive odor, and become alkaline in reaction. Growth is most luxuriant at a temperature of 24° C., but is plentiful also at 37° C. It is a facultative anaërobe and grows also in the absence of oxygen, but the proteus then loses its power of liquefying gelatin. It produces indol and phenol from peptone solutions. The proteus develops fairly well in urine, and decomposes urea into carbonate of ammonia.

Pathogenesis.—This bacillus is pathogenic for rabbits and guinea-pigs when injected in large quantities into the circulation, the abdominal cavity, or subcutaneously, producing death of the animals with symptoms of poisoning. Hauser has obtained the *Bacillus proteus* (*vulgaris*) from a case of purulent peritonitis, from purulent puerperal endometritis, and from a phlegmonous inflammation of

the hand. Brunner also reports similar infections in which this organism was found associated with pus cocci, and Charrin describes a case of pleuritis during pregnancy, in which the proteus was present and a foul-smelling secretion was produced. Death in this case, which ensued without further complication, is said to have been due probably to the poisonous products of the proteus.

An interesting example of pure toxæmia resulting from the toxin of the proteus is reported by Levy: While conducting some experiments on this organism he had an opportunity of making a bacteriological examination in the case of a man who died after a short attack of cholera morbus. From the vomited material and the stools he obtained a pure culture of the proteus; but the blood, collected at the autopsy, was sterile. In the meantime seventeen other persons who had eaten at the same restaurant were taken sick in the same way. Upon examination at the restaurant it was found that the bottom of the ice-chest in which the meat was kept was covered with a slimy, brown layer, which gave off a disagreeable odor. Cultures from this gave the proteus as the principal organism present. Injections into animals of the pure cultures produced similar symptoms as occurred in the human subjects.

Levy concludes that in so-called "flesh poisoning" bacteria of this group are chiefly concerned, and the pathogenic effects are due to toxic products evolved during their development.

Booker, from his extended researches into this subject, concludes that the proteus plays an important part in the production of the morbid symptoms which characterize cholera infantum. Proteus vulgaris was found in the alvine discharge in a large proportion of the cases examined by him, but was not found in the fæces of healthy infants. "The prominent symptoms in the cases of cholera infantum in which the proteus bacteria were found were drowsiness, stupor, and great reduction in flesh, more or less collapse, frequent vomiting and purging, with watery and generally offensive stools."

Next to the *Bacillus coli communis* the *Proteus vulgaris* appears to be the microörganism most frequently concerned in the etiology of pyelonephritis. In cases of cystitis and of pyelonephritis this bacillus is often found in pure cultures or associated with other bacteria. It probably gets into the bladder chiefly through catheterization. From the animal experiments of the authors above mentioned, simple injection of pure cultures of proteus into the bladder, without artificial suppression of urine, invariably produces severe cystitis. The fact that this organism grows in urine is sufficient to account for the extension of the purulent process finally to the kidneys.

The *Proteus vulgaris* is usually a harmless parasite when located in the mucous membrane of the nasal cavities. Here it only decomposes the secretions, with the production of a putrefactive odor. It is found occasionally in the discharge from cases of otitis media in connection with other bacteria.

CHAPTER XXXI.

GLANDERS BACILLUS (BACILLUS MALLEI).

THIS bacillus was discovered and proved to be the cause of glanders, by isolation in pure culture and inoculation into animals, by several bacteriologists almost at the same time (1882). The bacilli were first obtained in impure cultures by Bouchard, Capitan, and Charrin, and first accurately studied in pure culture by Loeffler and Schütz. They are present in the recent nodules in animals affected with glanders, and in the discharge from the nostrils, pus from the specific ulcers, etc., and occasionally in the blood.

Morphology.—Small bacilli with rounded or pointed ends, from nutrient agar cultures, 0.25μ to 0.5μ broad and from 1.5μ to 5μ long; usually single, but sometimes united in pairs, or growing out to long filaments, especially in potato cultures. The bacilli frequently break up into short, almost coccus-like elements (Fig. 131).

FIG. 131

Glanders bacilli. Agar culture. × 1100 diameters.

Staining.—The bacillus mallei *stains* with difficulty with the aniline colors, best when the aqueous solutions of these dyes are made feebly alkaline; it is decolorized by Gram's method. This bacillus presents the peculiarity of losing very quickly in decolorizing solutions the color imparted to it by the aniline staining solutions. For this reason it is difficult to stain in sections. Loeffler recommends his alkaline methylene-blue solution for staining sections, and for decolorizing, a mixture containing 10 c.c. of distilled water, 2 drops of strong sulphuric acid, and 1 drop of a 5 per cent. solution of oxalic acid; thin sections to be left in this acid solution for five seconds.

Biology.—An aërobic, non-motile bacillus, whose molecular movements are so active that they have often been taken for motility. It grows on various culture media at 37° C. Development takes place slowly at 22° C. and ceases at 43° C. The bacillus does not form spores. Exposure for ten minutes to a temperature of 55° C., or for five minutes to a 3 to 5 per cent. solution of carbolic acid, or for two minutes to a 1:5000 solution of mercuric chloride, destroys its vitality. As a rule, the bacilli do not grow after having been preserved in a desic-

cated condition for a week or two; in distilled water they are also quickly destroyed. It is doubtful whether the glanders bacillus finds conditions in nature favorable to a saprophytic existence.

Cultivation.—(For obtaining pure cultures see page 420.)—It grows well in the incubating oven on *glycerin agar*. Upon this medium at the end of twenty-four to forty-eight hours, whitish, transparent colonies are developed, which in six or seven days may attain a diameter of 7 or 8 mm. On *blood serum* a moist, opaque, slimy layer develops, which is of a yellowish-brown tinge. The growth on cooked *potato* is especially characteristic. At the end of twenty-four to thirty-six hours at 37° C. a moist, yellow, transparent layer develops; this later becomes deeper in color, and finally takes on a reddish-brown color, while the potato about it acquires a greenish-yellow tint. In *bouillon* the bacillus causes diffuse clouding, ultimately with the formation of a more or less ropy, tenacious sediment. It grows on media possessing a slightly acid reaction, and both with and without oxygen. *Milk* is coagulated with the production of acid.

Pathogenesis.—The bacillus of glanders is pathogenic for a number of animals. Among those which are most susceptible are horses, asses, guinea-pigs, cats, dogs, ferrets, moles, and field mice; sheep, goats, swine, rabbits, white mice, and house mice are much less susceptible; cattle are immune. Man is susceptible, and infection not infrequently terminates fatally.

When pure cultures of *Bacillus mallei* are injected into horses or other susceptible animals true glanders is produced. The disease is characterized in the horse by the formation of ulcers upon the nasal mucous membrane, which have irregular, thickened margins, and secrete a thin, virulent mucous; the submaxillary lymphatic glands become enlarged and form a tumor which is often lobulated; other lymphatic glands become inflamed, and some of them suppurate and open externally, leaving deep, open ulcers; the lungs are also involved, and the breathing becomes rapid and irregular. In farcy, which is a more chronic form of the disease, circumscribed swellings, varying in size from a pea to a hazel-nut, appear on different parts of the body, especially where the skin is thinnest; these suppurate and leave angry-looking ulcers with ragged edges, from which there is an abundant purulent discharge. The bacillus of glanders can easily be obtained in pure cultures from the interior of suppurating nodules and glands which have not yet opened to the surface, and the same material will give successful results when inoculated into susceptible animals. The discharge from the nostrils or from an open ulcer may contain comparatively few bacilli, and these being associated with other bacteria which grow more readily on the culture media than the bacillus mallei, make it difficult to obtain pure cultures from such material by the plate method. In that case, however, guinea-pig inoculations are useful.

Of test animals guinea-pigs and field mice are the most susceptible. In guinea-pigs subcutaneous injections are followed in four or five days by swelling at the point of inoculation, and a tumor with case-

ous contents soon develops; then ulceration of the skin takes place, and a chronic purulent ulcer is formed. The essential lesion is the granulomatous tumor, characterized by the presence of numerous lymphoid and epithelioid cells, among and in which are seen the glanders bacilli. The lymphatic glands become inflamed and general symptoms of infection are developed in from two to four weeks; the glands suppurate, and in males the testicles are involved; finally purulent inflammation of the joints occur, and death ensues from exhaustion. The formation of the specific ulcers upon the nasal mucous membrane, which characterizes the disease in the horse, is rarely seen when guinea-pigs are inoculated. In these the process is often prolonged or remains localized on the skin. They succumb more rapidly to intraperitoneal injection, usually in from eight to ten days, and in males the testicles are invariably affected.

Mode of Spread.—Glanders occurs as a natural infection only in horses and asses; the disease is occasionally communicated to man by contact with affected animals, usually by inoculation on an abraded surface of the skin. The contagion may also be received on the mucous membrane. Infection has sometimes been produced in bacteriological laboratories. In man, as in horses, an acute and chronic form of glanders may usually be recognized. The disease in human beings is fatal in about 60 per cent. of the cases. It is transmissible also from man to man. Washerwomen have been infected from the clothes of a patient. The infective material exists in the secretions of the nose, in the pus of glanders nodules, and frequently in the blood; it may occasionally be found in the secretions of glands not yet affected, as in the urine, milk, and saliva, and also in the fœtus of diseased animals (Bonome). From recent observations it appears that glanders is by no means an uncommon disease among horses, particularly in southern countries, sometimes taking a mild course and remaining latent for a considerable time. Horses apparently healthy, therefore, may possibly spread the disease.

Attenuation of virulence occurs in cultures which have been kept for some time, and inoculations with such cultures may give a negative result, or, when considerable quantities are injected, may produce a fatal result at a later date than is usual when small amounts of a recent culture are injected.

Immunity.—Attempts have been made to produce artificial immunity against glanders, but so far with unsatisfactory results. According to Strauss, by intravenous inoculations of small quantities of living bacilli, dogs may be protected against an injection of quantities which usually kill them. Fenger has found that animals inoculated with glanders bacilli react less powerfully to fresh injections; and that rabbits which have recovered from an injection of glanders are subsequently immune, the immunity lasting for from three to six weeks. Ladowski has obtained positive results also in rabbits and cats by intravenous injections of sterilized cultures. Other observers have reported not only the production of immunity, but also cures by the

use of *mallein*. This is prepared in the same way as tuberculin. It consists of the glycerinated bouillon in which the glanders bacilli have grown and which contains the products of their growth and activity. Concentrated mallein is produced by evaporating a six-weeks-old culture of the glanders bacillus in 5 per cent. glycerin nutrient veal bouillon to 10 per cent. of its original bulk. Some evaporate the culture fluid only to 50 per cent. The dose for diagnostic purposes in horses is 2½ c.c. of the unevaporated preparation.

Use of Guinea-pigs for Cultures and Diagnosis.—It is often difficult to demonstrate microscopically the presence of the bacillus of glanders in the nodules which have undergone purulent degeneration, in the secretions from the nostrils, or in the pus from the specific ulcers and suppurating glands. It is then necessary to make immediate cultures and also animal tests of these discharges by inoculating susceptible animals, as guinea-pigs and mice, and then from those to obtain a pure culture; but this requires time, and in clinical work it is of great importance for the diagnosis to be established as quickly as possible. With this view Strauss has prepared a method which is prompt and which has given very satisfactory results. This consists in introducing into the peritoneal cavity of a male guinea-pig some material or a culture from the suspected products. If it be a case of glanders, the diagnosis may usually be made within two to five days from the tumefaction of the testicles, which become swollen, and show evidences of pus formation. One objection to this method, however, is that occasionally from the injection of impure material, as in the nasal secretion, the animal may die of septicæmia; but if pure matter can be obtained, as from the lymphatic glands of the horse, this method is generally satisfactory. Sometimes the reaction is delayed or develops only to a moderate extent. Further tests must then be carried out with the cultures obtained from the tissues.

Diagnostic Use of Mallein.—The diagnosis of glanders in horses in which the usual symptoms of the disease have not yet manifested themselves, or in which it is suspected, may often be made by the use of mallein. Following an injection of mallein in a glanderous horse (best made about midnight) there will be a local reaction, and a general reaction with a rise of temperature. The temperature usually begins to rise three or four hours after the injection, and reaches its maximum between the tenth and twelfth hour. Sometimes, however, the highest point is not reached until fifteen or eighteen hours after the injection. This elevation of temperature is from 1.5° to 2° C. (2° to 3.5° F.), above the normal mean temperature. In a healthy animal the rise of temperature, as a rule, amounts to only a few tenths of a degree, but it may reach 1° C. The rise of temperature, however, should be considered always in connection with the general and local reactions. In a glanderous animal, after an injection of mallein, the general condition is more or less profoundly modified. The animal has a dejected appearance; the countenance is pinched and anxious, the hair is rough, the flank is retracted, the respirations are rapid, there

are often rigors, and the appetite is gone. In healthy animals the general symptoms do not occur. The local reaction around the point of injection in a glanderous animal is usually very marked. A few hours after the injection there appears a large, warm, tense, and very painful swelling, and running from this will be seen hot, sensitive lines of sinuous lymphatics, directed toward the neighboring lymphatic nodes. This œdema increases for twenty-four to thirty-six hours and persists for several days, not disappearing entirely for eight or ten days. In healthy animals, at the point of injection, mallein produces only a small œdematous tumor, and the œdema, instead of increasing, diminishes rapidly and disappears within twenty-four hours. The value of this test has been demonstrated by numerous experiments. There are some exceptions to the rule as described above, but they are infrequent, and mallein has been used with considerable success as a diagnostic aid in detecting the existence or absence of glanders in doubtful or obscure cases.

Agglutination Test for Glanders.—The test may be carried out according to the macroscopic or microscopic method.

Collection of the Blood.—In obtaining blood from horses a large-sized hypodermic needle which has been sterilized is inserted into the jugular vein which has been brought into view by pressing the thumb upon it from below; the blood is allowed to flow through the needle into a sterile tube or flask, 8 to 10 c.c. being sufficient.

In the case of human beings it is obtained by pricking the lobe of the ear or finger and collected in small capillary pipettes sealed at both ends. Care must be taken to keep the blood sterile.

Macroscopic Method.—The procedure of Meissner and Schütz with slight modifications is as follows: A forty-eight-hour glycerin agar culture of *Bacillus mallei* is washed off with normal salt solution, to which sufficient carbolic acid has been added to make a 5 per cent. solution. This is incubated for two hours at 60° C. then filtered and enough of the carbolized normal salt solution is added to give a slight milky appearance. This emulsion will keep for two or three weeks in the ice-box.

The serum is then made up into the required dilutions, such as 1:50, 1:100, etc. One c.c. of each dilution is pipetted into stoppered sterile serum tubes and an equal amount of the emulsion is added to each tube. The tubes are incubated at 37° C. for twenty-four to forty-eight hours.

If a reaction occurs the upper part of the fluid will be clear and a fine granular sediment will be found at the bottom or fine clumps clinging to the sides of the tube.

Meissner and Schütz use a culture of *Bacillus mallei* that has been recently passed through a guinea-pig, claiming that it agglutinates better than a culture grown for some time on artificial media. This is not in accordance with our experience. We have found that the more recently isolated culture, as in the case of *Bacillus typhi* and *Bacillus dysenteriæ*, shows much less agglutinability.

The Microscopic or Hanging Drop Method.—In this case a twenty-four-hour glycerin broth culture is used which has been heated to 60° C. for one minute, and the test is made as in the Widal for typhoid. The cover-glasses and slides must be sterilized and the hanging drops made carefully and quickly to avoid contamination. The slides are left at room temperature or at 22° C. for eighteen to twenty-four hours and then examined microscopically.

In this method the reaction can be observed earlier than in the tubes, that is as soon as agglutination occurs, and it is not necessary to wait for precipitation which at times takes place slowly.

The microscopic method gives a higher reading than the macroscopic method. This will include more horses which are doubtful, while on the other hand horses showing other symptoms of glanders will sometimes give a negative reaction with the gross method.

The limit of agglutination of the normal horse is 1:500, but many apparently healthy horses will agglutinate the *Bacillus mallei* in dilutions as high as 1:5000 and 1:10,000. The cause of this has not been fully decided. Such horses should be subjected to the mallein test from time to time, the possibility of a slight infection which may manifest itself at any time being kept in view. Very rarely a horse in the last stages of glanders will fail to give a reaction, but the clinical symptoms will be well-defined in such cases. So far, we have found the agglutination reaction valuable as a guide to the use of mallein, the facility with which it can be carried out admitting of the testing of a large number of horses suspected of having glanders or those having been exposed to the disease.

In human cases the reaction of 1:100 and above is considered positive, the normal blood not reacting above 1:50.

In very acute cases that run their course in a few days, the reaction may be entirely absent.

CHAPTER XXXII.

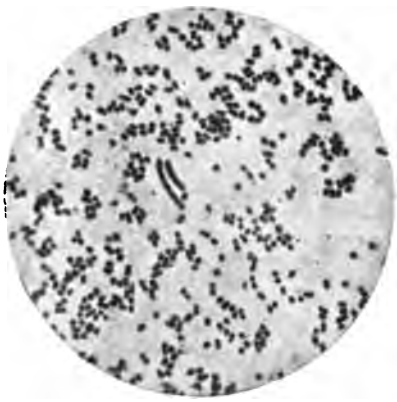
MICRO-ORGANISMS BELONGING TO THE HEMORRHAGIC SEPTICEMIA GROUP.

A number of bacilli of similar characteristics have been described as causing certain infectious diseases of lower animals, marked by the appearance of hemorrhagic areas throughout the body (hemorrhagic septicemia of Hueppe). The bacilli are short, bipolar-staining, non-motile, non-sporebearing organisms. They are Gram-negative and do not liquefy gelatin. They are found in rabbit septicemia, fowl cholera, swine plague, and a similar disease in cattle. The bacillus of bubonic plague seems to be closely related to the bacteria of this group, and Ricketts, who recently reported finding organisms similar to these in Rocky Mountain spotted fever and in typhus fever of Texas suggested that these three diseases be considered a group of human hemorrhagic septicemias.

BACILLUS OF BUBONIC PLAGUE (BACILLUS PESTIS).

HISTORICALLY we can trace the bubonic plague back to the third century. In Justinian's reign a great epidemic spread over the

FIG. 132



Bacillus pestis from agar culture.
× 1100 diam.

FIG. 133

Bacillus pestis from bouillon culture.
× 1100 diam.

Roman empire and before it terminated destroyed in many portions of the country nearly 50 per cent. of the people. The fourteenth century saw the whole of Europe stricken. Except for occasional cases, Europe and America have of late been free, but in India the disease has recently broken out in all its horrors so that at the present

time over 500,000 persons die annually from it. Among the most fatal forms of infection is that of the lungs. Pneumonic cases are not alone very serious, but they readily spread the infection. The bacillus exciting the disease was discovered simultaneously by Kitasato and Yersin (1894) during an epidemic of the bubonic plague in China. It is found in large numbers in the seropurulent fluid from the recent buboes characteristic of this disease and in the lymphatic glands; more rarely in the internal organs except in pneumonic cases when the lungs and sputum contain immense numbers, it occurs in the blood in acute hemorrhagic cases and shortly before death. It also occurs in malignant cases in the fæces of men and animals. The bacillus, as we have stated, is closely allied to the hemorrhagic septicæmia group.

FIG. 134

Involution forms on salt agar. (Kolle and Wassermann.)

Morphology.—The bacilli in smears from acute abscesses or infected tissues are, as a rule, short, thick rods with rounded ends. The central portion of the bacillus is slightly convex. When lightly stained the two ends are more colored than the middle portion. The bacilli are mostly single or in pairs. Bacilli in short chains occur at times. The length of the bacilli varies, but on the average is about 1.6μ (1.5μ to 1.7μ), breadth 0.5μ to 0.7μ. Besides the usual oval form, the plague bacillus has many exceptional variations which are characteristic of it. In smears, especially from old buboes, one looks for long bacilli with clubbed ends (similar to involution forms (Fig. 134), yeast-like forms, and bladder shapes. Some of these stain with difficulty. When obtained from cultures the bacilli present not only the forms already mentioned, but also long chains.

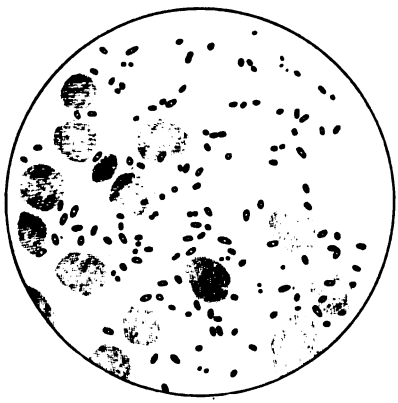
Staining.—They *stain* readily with the ordinary aniline dyes, and especially well with methylene blue, the ends being usually more deeply colored than the central portion; they do not stain by Gram's method.

Biology.—An aërobic, non-motile bacillus. Grows best at 30° to 35° C. Does not form spores. Grows on the usual culture media, which should have a slightly alkaline reaction. Does not liquefy gelatin. Grows well on *blood-serum* media. It grows rapidly on *glycerin agar*, forming a grayish-white surface growth. The bacilli appear, as a rule, as short, plump, oval bacilli, but a few present elongated thread forms which are very characteristic. In *bouillon* which is kept still a very characteristic appearance is produced, the

culture medium remaining clear while a pellicle forms on the surface from which projections sprout downward (stalactite formation) toward a granular or grumous deposit which forms on the walls and on the bottom of the tube. In bouillon and most fluid media the growth is in the form of short or medium chains of very short, oval bacilli, which look almost like streptococci.

Pathogenesis.—This bacillus is pathogenic for rats, mice, guinea-pigs, monkeys, rabbits, fleas, flies, and other insects, which usually die within two or three days after inoculation. Then at the point of inoculation is found a somewhat hemorrhagic infiltration and œdema, with enlargements of the neighboring lymph glands, hemorrhages into the peritoneal cavity, and parenchymatous congestion of the organs. The spleen sometimes shows minute nodules resembling miliary tubercles. Microscopically the bacilli are found in all the organs and in the blood. The disease is rapidly communicated from one animal to another through the bites of infected fleas, and thus its extension is facilitated. During epidemics, rats, mice, and flies, in large numbers, become infected and die, and the disease is frequently transmitted through them to man. The organism is found at times in the fæces of sick animals, in the dust of infected houses, and in the soil.

FIG. 135



Bacilli in smear from acutely inflamed gland.

Ground squirrels in California have been shown to be susceptible to infection and they are supposed to help spread the disease.

The virulence of the bacilli in cultures and in nature seems to vary considerably, and rapidly diminishes when grown on artificial media. The growth in cultures becomes more abundant after frequent transplantation. The virulence of the organism is increased by successive inoculation in certain animal species, and then its pathogenic properties for other species are less marked.

In man there are often subcutaneous hemorrhages in severe cases which gave the disease its name of "black death."

Immunity.—Yersin, Calmette, and Borrel have succeeded in immunizing animals against the bacillus of bubonic plague by the intravenous or intraperitoneal injection of dead cultures, or by repeated subcutaneous inoculation. They also succeeded in immunizing rabbits and horses, so that the serum afforded protection to small animals, after subcutaneous injection of virulent cultures, and even cured those which had been inoculated, if administered within twelve hours after injection. The serum has considerable antitoxic as well as bactericidal properties. More recently this serum has been applied

to the treatment of bubonic plague in man, with promising results. Experience has shown that the treatment is more efficacious the earlier the stage of the disease. When treatment is begun in the first day of the attack, fever and all alarming symptoms sometimes disappear with astonishing rapidity. In cases treated at a later stage larger doses of the serum are required, and even in the favorable cases suppuration of the buboes is not always prevented. In some of the early cases and in many of the rather late ones the serum fails. When the disease is far advanced the serum is powerless. For immunizing purposes the serum should be valuable, and a single injection would probably give protection for several weeks.

Vaccines.—Haffkine, in India, has applied his method of preventive inoculation to the bubonic plague, as he previously did with cholera, and apparently with equally good results. This method consists in an inoculation of dead cultures, and is essentially a protective rather than a curative treatment. It gives after six to ten days a considerable immunity, lasting a month or more. By means of these two methods of inoculation, along with strict quarantine regulations and the destruction of rats and fleas, it is to be hoped that this disease which, under the name of Black Death, once decimated the populations of the earth and which in the East still causes a great mortality may finally be greatly restricted.

Duration of Life Outside of the Body.—In cultures protected from the air and light the plague bacilli may live one year or more. In the bodies of dead rats they may live for two months. In sputum from pneumonic cases the bacilli lived ten days. Upon sugar sacks, food, etc., they may live six to fifteen days.

Resistance to Deleterious Influences.—The bacilli resemble the colon bacilli in their reaction to heat and disinfectants. Boiling for one to two minutes kills them. Carbolic acid, 5 per cent. solution, kills cultures in one minute, in 2½ per cent. in two minutes, etc.

Bacteriological Diagnosis.—When the lymph glands are acutely inflamed but not yet suppurated, cut down on one and make cultures on nutrient agar slanted in tubes. If pus has formed withdraw a little by means of the hypodermic needle. There should also be made smears from the suspected bubo, or in case of pneumonia from the sputum. If the patient is dead, cultures from the spleen and heart's blood are also taken when possible. Suspected animals, such as rats and mice, when freshly killed, are examined as in man; when decomposed, rats and guinea-pigs should be inoculated.

ROCKY MOUNTAIN SPOTTED FEVER.

Rocky Mountain spotted fever and typhus fever have this in common with bubonic plague, they are acute infectious diseases characterized by fever and a more or less hemorrhagic eruption. These two diseases have been especially studied for the last few years by Ricketts of Chicago and his associates. They began with Rocky Mountain spotted fever[1]. In this disease, some years ago, Wilson and

[1] Ricketts, H. T. Jour. Am. Med. Assoc., 1909, lii, 379.

Chowning thought they found protozoa similar to *Babesia* of Texas fever. Anderson was inclined to agree with them, but nobody else could find these bodies even in the original slides. These investigators proved, however, that rabbits are susceptible to the disease and that a tick of the genus Dermacentor carries the infection. Then Ricketts and Gomez made some very interesting studies on the disease. They found that guinea-pigs and monkeys are susceptible as well as rabbits, and they further found that in guinea-pigs and monkeys an attack of spotted fever produces a strong active inherited immunity characterized by a serum with high protective but low curative power, and that the production of the serum in the horse with the use of sero-vaccination in man may give practical results.

They found a moderate number of diplococcoid bodies in the blood of infected guinea-pigs and monkeys, and fever in man. They described these bodies as two small, lanceolate, chromatin-staining (Giemsa stain) bodies separated by a small amount of eosin-staining substance. They did not find the bodies in normal blood, but they state that, considering the complex morphology of the blood and the fact that they could get no culture, it could not yet be stated that these are micro-organisms. They found that the virus is transmitted by the infected female tick to her young through the eggs. If the larvæ from these eggs are allowed to feed upon normal guinea-pigs, these animals come down with the disease. Immense numbers of these apparent organisms are found in infected eggs and none were found at first in normal eggs. Afterward Ricketts found a few, but he thought these might be an avirulent species of the same organism.

The salivary glands, alimentary, sac and ovaries of infected female ticks are swarming with these bodies, while normal ticks seem to have none. Lastly, Ricketts found that these bodies agglutinate with specific serum, 1 to 300 dilution. These bodies resemble the bacilli belonging to the hemorrhagic septicæmia group of organisms.

TYPHUS FEVER.

Nicolle (July, 1909)[1] had showed that old world typhus can be transmitted to the chimpanzee and from this to the macacus with typical eruption in each case. He also showed that the disease is transmitted by the ordinary body louse (*Pediculus vestimenti*). He was not able to transmit from monkey to monkey.

Anderson and Goldberger (December, 1909)[2] were the first to transmit the typhus fever of Mexico (tabardillo) to monkeys. They were able to transmit directly from human beings to the macacus and capuchin.

Ricketts and Walker (February, 1910)[3] also found that the macacus was directly susceptible to the disease. They based their diagnosis

[1] Compt. rend. Acad. Sci., cxlix.
[2] Public Health Reports (U. S.), xxiv, Nos. 50 and 52.
[3] Jour. of Am. Med. Assoc., liv, 463, 1304, 1373.

chiefly upon a rather indefinite fever, and, in most cases, somewhat distinct symptoms of illness.

Their conclusions are as follows:

1. It seems that *M. rhesus* can be infected with tabardillo invariably by the injection of virulent blood from man taken on eighth to tenth days of fever. The blood should be diluted with salt solution.

2. Attempts to maintain typhus in the monkey by passage through other monkeys were not successful.

3. The monkey may pass through an attack of typhus so mild that it cannot be recognized clinically. Vaccination results.

4. The immunity test is a reliable proof of the previous occurrence or non-occurrence of typhus at least within a period of one month.

5. Typhus was transmitted to the monkey by the bite of the louse (*Pediculus vestimenti*) in second experiment, the lice in one instance deriving their infection from man and in another from the monkey.

6. Another monkey was infected by typhus through the introduction of the fæces and abdominal contents of infected lice into small incisions.

7. In stained (Giemsa) preparations of blood of patients taken from seventh to twelfth days of disease we invariably found a few short bacilli (300 to 2,000 bacilli to 0.01 c.c. of blood) which have roughly the morphology of those which belong to the hemorrhage septicæmia group.

8. In moist preparation similar forms have been seen in all cases. No motility observed. No cultures could be obtained.

9. Dejecta and organisms of many lice were examined and similar stained bodies have been found in large numbers in infected lice, occasionally in non-infected ones.

CHAPTER XXXIII.

THE ANTHRAX BACILLUS AND THE PATHOGENIC ANAEROBES.

BACILLUS ANTHRACIS.

ANTHRAX is an acute infectious disease which is very prevalent among animals, particularly sheep and cattle. Geographically and zoölogically it is the most widespread of all infectious disorders. It is much more common in Europe and in Asia than in America. The ravages among herds of cattle in Russia and Siberia and among sheep in certain parts of France, Hungary, Germany, Persia, and India are not equalled by any other animal plague. Local epidemics have occasionally occurred in England, where it is known as splenic fever. In this country the disease is rare. In infected districts the greatest losses are incurred during the hot months of summer.

The disease also occurs in man as the result of infection, either through the skin, the intestines, or in rare instances through the lungs. It is found in persons whose occupations bring them into contact with animals or animal products, as stablemen, shepherds, tanners, butchers, and those who work in wool and hair. Two forms of the disease have been described—the external anthrax, or malignant pustules, and the internal anthrax, of which there are intestinal and pulmonary forms, the latter being known as "wool-sorters' disease."

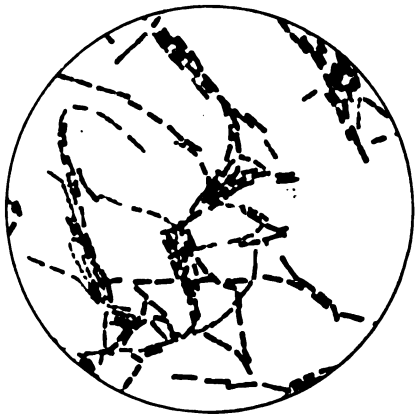
Owing to the fact that anthrax was the first infectious disease which was shown to be caused by a specific microörganism, and to the close study which it received in consequence, this disease has probably contributed more to our general knowledge of bacteriology than any other infectious malady.

Pollender in 1849 observed that the blood of animals suffering from splenic fever always contained minute rod-shaped bacteria. Davaine in 1863 announced to the French Academy of Sciences the results of his inoculation experiments, and asserted the etiological relations of the microörganism to the disease, with which his investigation showed it to be constantly associated. For a long time this conclusion was energetically opposed until, in 1877, Koch, Pasteur, and others established its truth by obtaining the bacillus in pure cultures, and showing that the inoculation of these cultures produced anthrax in susceptible animals as certainly as did the blood of an animal recently dead from the disease.

Morphology.—Slender, cylindrical, non-motile rods, having a breadth of 1μ to 1.25μ, and ranging from 2μ or 3μ to 20μ or 25μ in length. Sometimes short, isolated rods are seen, and, again, shorter

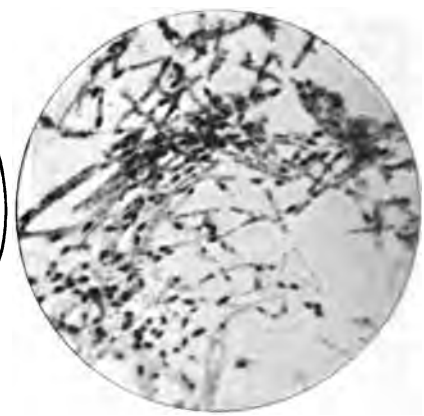
or longer chains or threads made up of several rods joined end to end. In suitable culture media very long, flexible filaments may be observed, which are frequently united in twisted or plaited cord-like bundles. (See Fig. 136 and Fig. 137 and Fig. 5, p. 11.) These filaments in hanging-drop cultures, before the development of spores, appear to be homogeneous or nearly so; but in stained preparations they are seen to be composed of a series of rectangular, deeply stained segments. When obtained directly from the blood of an infected animal the free ends of the rods are slightly rounded, but

FIG. 136



Anthrax bacillus. × 900 diameters. Agar culture.

FIG. 137



Spores heavily stained (in specimen red) Bodies of disintegrating bacilli faintly stained (in specimen blue). × 1000 diameters.

those coming in contact with one another are quite square. In cultures the ends are seen to be a trifle thicker than the body of the cell and somewhat concave, giving the appearance of joints of bamboo. At one time much stress was laid upon these peculiarities as distinguished marks of the anthrax bacillus; but it has been found that they are the effects of artificial cultivation and not necessarily characteristic of the organism under all conditions. Another peculiarity of this bacillus is that it is enclosed in a transparent envelope or capsule, which in stained preparations (from albuminous material) may be distinguished by its taking on a lighter stain than the deeply stained rods which it surrounds.

Under favorable conditions in cultures spores are developed in the bacilli. These spores are elliptical in shape and about one and a half times longer than broad. They first appear as small, refractive granules distributed at regular intervals, one in each rod. As the spore develops the mother-cell becomes less and less distinct, until it disappears altogether, the complete oval spore being set free by its dissolution. (See Fig. 137.) Irregular sporulation sometimes takes place, and occasionally there is no spore formation, as in varieties of non-spore-bearing anthrax.

Staining.—The anthrax bacillus *stains* readily with all the aniline colors, and also by Gram's method, when not left too long in the decolorizing solution. In sections good results may be obtained by the employment of Gram's solution in combination with carmine, but when only a few bacilli are present this method is not always reliable, as some of the bacilli are generally decolorized.

Biology.—The anthrax bacillus grows easily in a variety of nutrient media at a temperature from 18° to 43° C., 37° C. being the most favorable temperature. Under 12° C. no development takes place, as a rule, though by gradually accustoming the bacillus to a lower temperautre it may be induced to grow under these conditions. Under 14° C. and above 43° C. spore formation ceases. The lower limit of growth and of sporulation is of practical significance in determining the question whether development can occur in the bodies of animals dead from anthrax when buried at certain depths in the earth. Kitasato has shown that at a depth 1.5 metres the earth in July has a temperature of 15° C. at most, and that under these conditions a scanty sporulation of anthrax bacilli is possible, but that at a depth of 2 metres sporulation no longer occurs. The anthrax bacillus is aërobic—that is, its growth is considerably enhanced by the presence of oxygen—but it grows also under anaërobic conditions, as is shown by its growth at the bottom of the line of puncture in stick cultures in solid media; but under these conditions it no longer produces the peptonizing ferment which it does with free access of air. Furthermore, the presence of oxygen is absolutely necessary for the formation of spores, while carbonic acid gas retards sporulation. This explains, perhaps, why sporulation does not take place within the animal body either before or after death.

It is also capable of leading a saprophytic existence. The bacillus is non-motile.

Growth in Gelatin.—In *gelatin-plate cultures*, at the end of twenty-four to thirty-six hours at 24° C., small, white, opaque colonies are developed, which, under a low-power lens, are seen to be dark gray in the centre and surrounded by a greenish, irregular border, made up of wavy filaments. As the colony develops on the surface of the gelatin these wavy filaments spread out, until finally the entire colony consists of a light gray, tangled mass, which has been likened to a Medusa head (Fig. 138).

At the same time the gelatin begins to liquefy, and the colony is soon surrounded by the liquefied medium, upon the surface of which it floats as an irregular, white pellicle. In *gelatin-stick cultures* at first development occurs along the line of puncture as a delicate white thread, from which irregular, hair-like projections soon extend perpendicularly into the culture medium, the growth being most luxuriant near the surface, but continuing also below. At the end of two or three days liquefaction of the medium commences at the surface and gradually progresses downward.

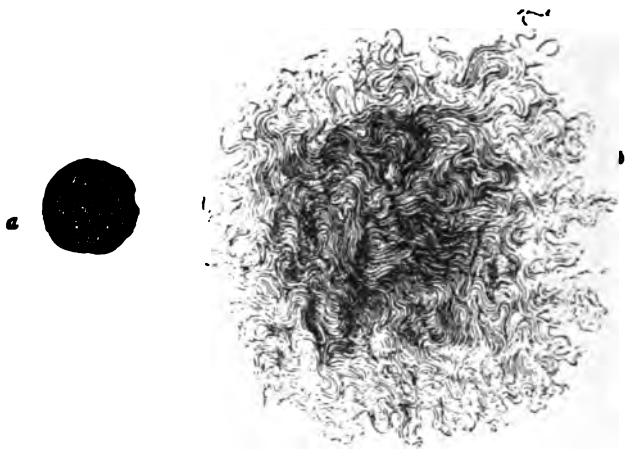
Growth on Agar.—The growth on *agar-plate cultures* in the incu-

bator at 37° C. is similar to that on gelatin, and is still more characteristic and beautiful in appearance. A grayish-white layer is formed on the surface within twenty-four hours, which spreads rapidly and is seen to be made up of interlaced threads.

Growth in Bouillon.—The growth is characterized by the formation of flaky masses, which sink as a sediment to the bottom of the tube, leaving the supernatant liquid clear.

Spore formation, as already noted, only takes place in the presence of oxygen, and at a temperature of 15° to 43° C. There is no development of spores at a greater depth than 1.5 metres in the earth, or in

FIG. 138



Colonies of bacillus anthracis upon gelatin plates: *a*, at the end of twenty-four hours; *b*, at the end of forty-eight hours. × 80. (F. Flügge.)

the bodies of living or dead animals; but spores may be found in the fluids containing the bacilli when these come in contact with the air, as in bloody discharges from the nostrils or from the bowels of the dead animal.

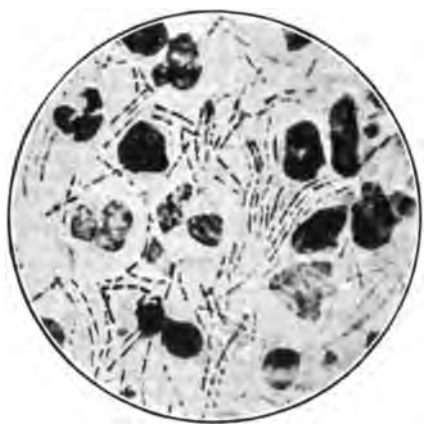
There are certain non-spore-bearing species of anthrax. Sporeless varieties have also been produced artificially by cultivating the typical anthrax bacillus under certain conditions, among which may be mentioned the addition of antiseptics, as carbolic acid, and of continued high temperature (43° C). Varieties differing in their pathogenic power may also be produced artificially. Pasteur produced an "attenuated virus" by keeping his cultures for a considerable time before replanting them upon fresh soil.

Anthrax cultures containing spores retain their vitality for years; in the absence of spores the vitality is much more rapidly lost. When grown in liquids rich in albumin the bacilli attain a considerable degree of resistance; thus dried anthrax blood has been found to retain its virulence for sixty days, while dried bouillon cultures only did so for twenty-one days. Dried anthrax spores may be preserved

for many years without losing their vitality or virulence. They also resist a comparatively high temperature. Exposed in dry air they require a temperature of 140° C. maintained for three hours to destroy them; but suspended in a liquid they are destroyed in four minutes by a temperature of 100° C.

Pathogenesis.—The anthrax bacillus is pathogenic for cattle, sheep (except the Algerian race), horses, swine, mice, guinea-pigs, and rabbits. Rats, cats, dogs, chickens, owls, pigeons, and frogs are but little susceptible to infection. Small birds—the sparrow particularly—are somewhat susceptible. Man, though subject to local infection and occasionally to internal forms of the disease, is not as susceptible as some of the lower animals.

FIG. 139



Section of liver from mouse dead of anthrax septicæmia. × 1000 diameters. (From Itserott and Niemann).

In susceptible animals the anthrax bacillus produces a true septicæmia. Among test animals mice are the most susceptible, succumbing to very minute injections of a slightly virulent virus; next guinea-pigs, and lastly rabbits, both of these animals dying after inoculation with virulent bacilli. Infection is most promptly produced by introduction of the bacilli into the circulation or the tissues, but inoculation by contact with wounds on the skin also causes infection. It is difficult to produce infection by the ingestion even of spores; but it may readily be caused by inhalation, particularly of spores.

Subcutaneous injections of these susceptible animals results in death in from one to three days. Comparatively little local reaction occurs immediately at the point of inoculation, but beyond this there is an extensive œdema of the tissues. Very few bacilli are found in the blood in the larger vessels, but in the internal organs, and especially in the capillaries of the liver, the kidneys, and the lungs, they are present in great numbers. In some places, as in the glomeruli of the kidneys, the capillaries will be seen to be stuffed full of bacilli, and hemorrhages, probably due to rupture of capillaries by the mechanical pressure of the bacilli which are developing within them, may occur. The pathological lesions in animals infected by anthrax are not marked except in the spleen, which, as in other forms of septicæmia, is greatly enlarged.

Occurrence in Cattle and Sheep.—Cattle and sheep are affected chiefly with the intestinal form of anthrax, infection in these animals commonly resulting from the ingestion of food containing spores. The bacillus itself, in the absence of spores, is quickly de-

stroyed by the gastric juice. The disease usually takes a rapid course, and the mortality is high—70 to 80 per cent. The pathological lesions consist of numerous ecchymoses, enlargement of the lymphatic glands, serous, fatty, and hemorrhagic infiltration of the mediastinum and mesentery, of the mucous membranes of the pharynx and larynx, and particularly of the duodenum, great enlargement of the spleen, and parenchymatous changes in the lymphatic organs. The blood is very dark and tar-like. Bacilli are present, especially in the lymph spaces, in enormous masses.

Sheep are also subject to external anthrax, infection taking place by way of the skin; cattle are seldom infected in this way. At the point of inoculation there develops a hard, circumscribed boil—the so-called anthrax carbuncle; or there may be diffuse œdema with great swelling of the parts. When death occurs the appearances are similar to those in intestinal anthrax, except that the duodenum is usually less affected; but in all cases metastasis occurs in various parts of the body, brought about, no doubt, by previous hemorrhages.

Occurrence in Man.—The disease does not occur spontaneously in man, but always results from infection, either through the skin, the intestines, or occasionally by inhalation through the lungs. It is usually produced by cutaneous infection through inoculation of exposed surfaces—the hands, arms, or face. Infection of the face or neck would seem to be the most dangerous, the mortality in such cases being 26 per cent., while infection of the extremities is rarely fatal.

External anthrax in man is similar to this form of the disease in animals. There are two forms: malignant pustule or carbuncle, and, less commonly, malignant anthrax œdema.

In malignant pustule, at the site of inoculations, a small papule develops, which becomes vesicular. Inflammatory induration extends around this, and within thirty-six hours there is a dark brownish eschar in the centre, at a little distance from which there may be a series of small vesicles. The brawny induration may be extreme. There may also be considerable œdema of the parts. In most cases there is no fever; or the temperature at first rises rapidly and the febrile phenomena are marked. Death may take place in from three to five days. In cases which recover the symptoms are slighter. In the mildest form there may be only slight swelling.

Malignant anthrax œdema occurs in the eyelids, and also in the head and neck, sometimes the hand and arm. It is characterized by the absence of the papule and vesicle forms, and by the most extensive œdema. The œdema may become so intense that gangrene results; such cases usually prove fatal.

The bacilli are found on microscopic examination of the fluid from the pustule shortly after infection; later the typical anthrax bacilli are often replaced by involution forms. In this case resort may be had to cultures, animal inoculation, or examination of sections of the extirpated tumor. The bacilli are not present in the

blood until just before death. Along with the anthrax bacilli pus cocci are often found in the pustule penetrating into the dead tissue.

Internal anthrax is much less common in man; it does, however, occur now and then. There are two forms of this: the intestinal form, or mycosis intestinalis, and the pulmonic form, or wool-sorters' disease.

Intestinal anthrax is caused by infection through the stomach and intestines, and results probably from the eating of raw flesh or unboiled milk of diseased animals. That the eating of flesh from infected animals is comparatively harmless is shown by Gerlief, who states that of 400 persons who are known to have eaten such meat not one was affected with anthrax. On the other hand, an epidemic of anthrax was produced among wild animals, according to Jansen, by feeding them on infected horse-flesh. It is evident, therefore, that there is a possibility of infection being caused in this way. The recorded cases of intestinal anthrax in man have occurred in persons who were in the habit of handling hides, hair, etc., which were contaminated with spores; in those who were conducting laboratory experiments, and rarely it has been produced by the ingestion of food, such as raw ham and milk. The symptoms produced in this disease are those of intense poisoning: chill, followed by vomiting, diarrhœa, moderate fever, and pains in the legs and back. The pathological lesions are similar to those described in animals.

Wool-sorters' disease, or pulmonic anthrax, is found in large establishments in which wool and hair are sorted and cleansed, and caused by the inhalation of dust contaminated with anthrax spores. The attack comes on with chills, prostration, then fever. The breathing is rapid, and the patient complains of pain in the chest. There may be a cough and signs of bronchitis. The bronchial symptoms in some instances are pronounced. Death may occur in from two to seven days. The pathological changes produced are swelling of the glands of the neck, the formation of foci of necrosis in the air passages, œdema of the lungs, pleurisy, bronchitis, enlargement of the spleen, and parenchymatous degenerations.

Prophylaxis against Anthrax Infection.—Numerous investigations have been undertaken with the object of preventing infection from anthrax. The efforts of Pasteur to effect immunity in animals by preventive inoculations of "attenuated virus" of the anthrax bacillus opened a new field of productive original research. Following in his wake many others have devised methods of immunization against anthrax infection; but the one adopted by Pasteur, Chamberland, and Roux has alone been practically employed on a large scale. According to these authors, two anthrax cultures of different degrees of virulence attenuated by cultivation at 42° to 43° C., are used for inoculation. Vaccine No. 1 kills mice, but not guinea-pigs; vaccine No. 2 kills guinea-pigs, but not rabbits. The animals to be inoculated —viz., sheep and cattle—are first given a subcutaneous injection of one to several tenths of a cubic centimetre of a four-day-old bouillon

culture of vaccine Nº. 1; after ten to twelve days they receive a similar dose of vaccine No. 2. Prophylactic inoculations given in this way have been widely employed with apparently good results.

Bacterial Cultures for Diagnosis.—The detection of the anthrax bacillus is ordinarily not difficult, as this organism presents morphological, biological, and pathogenic characteristics which distinguish it from all other bacteria. In the later stages of the disease, however, the bacilli may be absent or difficult to find, and cultivation on artificial media and experimental inoculation in animals are not always followed by positive results. Even in sections taken from the extirpated pustule it is sometimes difficult to detect the bacilli. In such cases only a probable diagnosis of anthrax can be made. It should be remembered that the bacilli are not found in the blood until shortly before death, and then only in varying quantity; thus blood examinations often give negative results, though the bacilli may be present in large numbers in the spleen, kidneys, and other organs of the body. The suspected material should be streaked over nutrient agar in Petri plates and inoculated in mice.

Differential Diagnosis.—Among other bacteria which may possibly be mistaken for anthrax bacilli are *Bacillus subtilis* and the bacillus of maglinant œdema. The former is distinguished by its motility, by various cultural peculiarities, and by being non-pathogenic. The latter differs from the anthrax bacillus in form and motility, in being decolorized by Gram's solution, in being a strict anaërobe, and in various pathogenic properties.

The diagnosis of internal anthrax in man is by no means easy, unless the history points definitely to infection in the occupation of the individual. In cases of doubt cultures should be made and inoculations performed in animals.

BACILLUS ANTHRACIS SYMPTOMATICI (BACILLUS OF SYMPTOMATIC ANTHRAX).

Like the bacilli of anthrax and of malignant œdema, both of which it resembles in other respects also, the bacillus of symptomatic anthrax is an inhabitant of the soil. It is found as the chief cause of the disease in animals—principally cattle and sheep—known as "black leg," "quarter evil," or symptomatic anthrax (rauschbrand, German; charbon symptomatique, French), a disease which is characterized by a peculiar emphysematous swelling of the subcutaneous tissues and muscles, especially over the quarters. Clinically it is sometimes confused with anthrax.

Morphology.—Bacilli having rounded ends, from 0.5μ to 0.6μ broad and from 3μ to 5μ long; mostly isolated; also occurring in pairs, joined end-to-end, but never growing out into long filaments, as the anthrax bacilli in culture and the bacilli of malignant œdema in the bodies of animals are frequently seen to do. In the hanging drop the bacilli are observed to be actively motile, and in stained prepara-

tions flagella may be demonstrated surrounding the periphery. The spores are elliptical in shape, usually thicker than the bacilli, lying near the middle of the rods, but rather toward one extremity. This gives to the bacilli containing spores a somewhat spindle shape.

Stains with the ordinary aniline dyes, but not with Gram's method or only with difficulty and after long treatment or intense colors.

Biology.—Like the bacillus of malignant œdema, this is a strict anaërobe, and cannot be cultivated in an atmosphere in which oxygen is present. It grows best under hydrogen, and does not grow under carbonic acid. This bacillus develops at the room temperature in the usual culture media, in the absence of oxygen, but it grows best in those to which 1.5 to 2 per cent. of glucose or 5 per cent. of glycerin has been added.

FIG. 140

Bacilli of symptomatic anthrax, showing spores. (After Zettnow.)

Growth on Agar.—The colonies on agar are somewhat more compact than those of malignant œdema, but they also send out projections very often. In *agar-stick cultures*, in the incubator, growth occurs after a day or two also some distance below the surface, and is accompanied by the production of gas and a peculiar disagreeable acid odor.

Pathogenesis.—The bacillus of symptomatic anthrax is pathogenic for cattle (which are immune against malignant œdema), sheep, goats, guinea-pigs, and mice; horses, asses, and white rats, when inoculated with a culture of this bacillus, present only a limited reaction; and rabbits, swine, dogs, cats, chickens, ducks, and pigeons are, as a rule, naturally immune to the disease. The guinea-pig is the most susceptible of test animals. When susceptible animals are inoculated subcutaneously with pure cultures of this organism, or with spores attached to a silk thread, or with bits of tissue from the affected parts of another animal dead of the disease, death ensues in from twenty-four to thirty-six hours. At the autopsy a bloody serum is found in the subcutaneous tissues, extending from the point of inoculation over the entire surface of the abdomen, and the muscles present a dark red or black appearance, even more intense in color than in malignant œdema, and there is a considerable development of gas. The lymphatic glands are markedly hyperæmic.

The disease occurs chiefly in cattle, more rarely in sheep and goats; horses are not attacked spontaneously—*i. e.*, by accidental infection. In man infection has never been produced, though ample opportunity by infection through wounds in slaughter-houses and by ingestion of infected meat has been given. The usual mode of natural infection by symptomatic anthrax is through wounds which penetrate not only

the skin, but the deep, intercellular tissues; some cases of infection by ingestion have been observed. The pathological findings present the conditions above described as occurring in the experimental infection.

Distribution Outside of the Body.—Symptomatic anthrax, like anthrax and malignant œdema, is a disease of the soil, but it shows a more limited endemic distribution than the former, and is differently distributed over the earth's surface than the second of these diseases, being confined especially to places over which infected herds of cattle have been pastured. It is doubtful whether the bacilli are capable of development outside of the body like anthrax. In the form of spores, however, reproduction may take place; by contamination with these, through deep wounds acquired by animals in infected pastures, the disease is spread.

Toxins.—Under favorable conditions extracellular toxins are formed so that the filtrate of cultures is very poisonous. Injections of the toxin into animals excite the production of antitoxins.

Differential Diagnosis.—The principal points of differentiating this bacillus from the bacillus of malignant œdema, which it closely resembles, are: it is smaller; it does not develop into long threads in the tissues; it is more actively motile, and forms spores more readily in the animal body than does the bacillus of malignant œdema. It is pathogenic for cattle, while malignant œdema is not; and swine, dogs, rabbits, chickens, and pigeons, which are readily infected with malignant œdema, are not, as a rule, susceptible to symptomatic anthrax.

Preventive Inoculations.—It is well known to veterinarians that recovery from one attack of symptomatic anthrax protects an animal against a second infection. Artificial immunity to infection can also be produced in various ways: by inoculations with cultures which have been kept for a few days at a temperature of 42° to 43° C. and have thus lost their original virulence, or by inoculations of filtered cultures, or of cultures sterilized by heat. For the production of immunity in cattle it is advised to use a dried powder of the muscles of animals which have succumbed to the disease, and which have been subjected to a suitable temperature to ensure attenuation of the virulence of the spores contained therein. Two vaccines are prepared, as in anthrax—a stronger vaccine by exposing a portion of the powder to a temperature of 85° to 90° C. for six hours, and a weaker vaccine by exposing it for the same time to a temperature of 100° to 104° C. Inoculations are made with this attenuated virus into the end of the tail—first the weaker and later the stronger. These give rise to a local reaction of moderate intensity, and the animal is subsequently immune from the effects of the most virulent material and from the disease. Fourteen days are allowed to elapse between the two inoculations. The results obtained from this method of preventive inoculation seem to have been very satisfactory. According to the statistics, including many thousand cattle treated, the

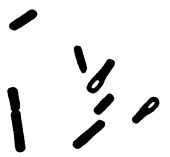
mortality, which among 22,300 non-inoculated cattle was 2.20 per cent., has been reduced to 0.16 per cent. in 14,700 animals inoculated. When danger of immediate infection exists, it is advisable to inject some antitoxin with the vaccine. This lessens the reaction and gives immediate immunity.

If an antitoxic serum is at hand it should be given in cases seen early in the disease.

THE GROUP OF MALIGNANT ŒDEMA BACILLI.

This group is widely distributed, being found in the superficial layers of the soil, in putrefying substances, in foul water, and by invasion from the intestine, in the blood of animals which have been suffocated. One such organism was discovered (1877) by Pasteur in animals after infection with putrid flesh, and named by him "vibrion septique." He recognized its anaërobic nature, but did not obtain it in pure culture. Koch and Gaffky (1881) carefully studied this microörganism, described it in detail, and gave it the name "*Bacillus œdematis maligni*" (Fig. 141).

Fig. 141



Bacilli of malignant œdema.

In earlier times infection of man was quite often, now only occasionally, produced. This bacillus belongs to a group which have lateral flagellæ, produce oval spores, and grow only anaërobically.

Morphology.—The œdema bacillus is a rod of from 0.8μ to 1μ in width, and of very varying length, from 2μ to 10μ or more, according to the conditions of its cultivation and growth. It is usually found in pairs, joined end to end, but may occur in chains or long filaments. It forms spores, and these are situated in or near the middle of the body of the rods. Exceptionally the spores are near the ends (see Fig. 141). The spores vary in length and are oval in form, being often of greater diameter than the bacilli, to which they give a more or less oval shape.

The bacilli *stain* readily by the usual aniline colors employed, but are usually decolorized by Gram's method. Freytag found that very young cultures were stained, while older ones were decolorized.

Biology.—A strictly anaërobic, liquefying, motile bacillus. Forms spores which are very resistant. It grows in all the usual culture media in the absence of oxygen. Development takes place at 20° C., but more rapidly and abundantly at 37° C.

Growth in Gelatin.—This bacillus may be cultivated in ordinary nutrient gelatin, but the growth is more abundant in *glucose gelatin* containing 1 or 2 per cent. of glucose. After two or three days small, almost transparent, circular colonies appear ½ to 1 mm. in diameter. Later, as liquefaction increases, the colonies become grayish and then confluent. Gas bubbles are formed and the gelatin liquefies.

Growth on Agar.—On agar plates the colonies appear as dull, whitish points, irregular in outline, and when examined under a low-power lens are seen to be composed of a dense network of interlacing threads, radiating irregularly from the centre toward the periphery.

Blood serum is rapidly liquefied, with the production of gas. Cultures of the malignant œdema bacillus give off gas with a peculiar, disagreeable odor.

Resistance.—The spores are very resistant and because of this the soil remains infected.

Pathogenesis.—The bacillus of malignant œdema is especially pathogenic for mice, guinea-pigs, and rabbits, although man, horses, dogs, goats, sheep, calves, pigs, chickens, and pigeons are also susceptible. A small quantity of a pure culture injected beneath the skin of a susceptible animal gives rise to an extensive hemorrhagic œdema of the subcutaneous connective tissue, which extends over the entire surface of the abdomen and thorax, causing hyperæmia and redness of the superficial muscles. No odor is developed, and there is little, if any, production of gas. In infection with garden earth, owing to the presence of associated bacilli, the effused serum is frothy from the development of gas and possesses a putrefactive odor. The disease, in natural infection caused by the contamination of wounds with earth or fæces, runs the course above described. Simple abrasion of the skin is not sufficient to produce infection; owing to the bacillus being capable only of an anaërobic existence, the poison must penetrate deep into the tissues. Malignant œdema is confined mostly to the domestic animals, the horse, sheep, cattle, and swine, but cases have also been reported in man.

Animals which recover from malignant œdema are subsequently immune. Artificial immunity may be induced in guinea-pigs by injecting filtered cultures of the malignant œdema bacillus in harmless quantities.

In man the chief symptom is the sudden appearance of subcutaneous œdematous swelling accompanied by high fever. In light cases this remains circumscribed; in severe cases it spreads widely and the case ends fatally. Appreciable quantities of gas usually fail. Autopsy shows a serous or hemorrhagic infiltration of the subcutaneous tissues and intramuscular connective tissue. In the inflamed tissue the bacilli with and without spores are found.

Prevention.—Most cases are produced by injecting subcutaneously albuminous fluids infected by the bacilli. Care should be taken that fluids to be injected do not become infected by dust or dirt.

BACILLUS AEROGENES CAPSULATUS (BACILLUS WELCHII).

This bacillus was found by Welch in the blood vessels of a patient suffering with aortic aneurysm; on autopsy, made in cool weather, eight hours after death, the vessels were observed to be full of gas bubbles. Since then it has been found in a number of cases in which gas has developed from within sixty hours of death until some hours after death. External cutting operations on the urethra and operations upon the uterus have been followed in a number of cases by infection. It has been found in ovarian abscesses and in infections of

the genito-urinary tract. These cases are, as a rule, marked by delirium, rapid pulse, high temperature, and the development of emphysema and discoloration of the diseased area or of marked abdominal distention when the peritoneal cavity is involved. This bacillus is present, as a rule, in the intestinal canal of man and animals and is apt to be found in the dust of hospitals and elsewhere. Herter[1] has shown that it is present in excessive numbers in certain diseases of the digestive tract. These cases are apt to develop anæmia.

Morphology.—Straight or slightly curved rods, with rounded or sometimes square-cut ends; somewhat thicker than the anthrax bacilli and varying in length; occasionally long threads and chains are seen. The bacilli in the animal body, and sometimes in cultures, are enclosed in a transparent capsule. Spores are usually absent in the tissues and often in cultures. Dunham showed that the culture isolated by Welch formed spores when grown on blood serum. Some strains since isolated make spores readily. It is possible that these differences may be due to the fact of there being several strains.

Biology.—An anaërobic, non-motile, non-liquefying bacillus. Different strains of this bacillus vary in their tendency to make spores. It is stained by Gram, but is more easily decolorized than many bacteria. Growth is rapid at 37° C., in the usual culture media in the absence of oxygen, and is accompanied by the production of gas. *Nutrient gelatin* is not liquefied by the growth of this bacillus, but it is gradually peptonized. If *agar* colonies are developed which are from 1 to 2 mm. or more in diameter, grayish-white in color, and in the form of flattened spheres, ovals, or irregular masses, beset with hair-like projections. *Bouillon* is diffusely clouded, and a white sediment is formed. *Milk* becomes acidified and coagulated, then partially digested, giving a worm-eaten appearance to the clot.

Pathogenesis.—Usually non-pathogenic in healthy animals, although Dunham found that the bacillus taken freshly from human infection is sometimes very virulent. When quantities up to 2.5 c.c. of fresh bouillon cultures are injected into the circulation of rabbits and the animals killed shortly after the injection, the bacilli develop rapidly, with an abundant formation of gas in the blood vessels and organs, especially the liver. This procedure is one of the best methods of obtaining the bacilli: The material suspected to contain the bacillus alone or associated with other bacteria is injected intravenously into rabbits, which are killed five minutes later and kept at 37° C. for sixteen hours, and cultures made from the liver and heart's blood.

It is suggested by Welch that in some of the cases in which death has been attributed to the entrance of air into the veins the gas found at the autopsy may not have been atmospheric air, but may have been produced by this or some similar microörganism entering the circulation and developing shortly before and after death. The same may be true for gas in the uterine cavity.

[1] Journal of Biolog. Chem., 1906, ii., page 1.

BACILLUS ENTERITIDIS SPOROGENES.

This very closely resembles B. Welchii. It produces spores readily. Klein considers that when taken in milk it may produce diarrhœa. This is disputed by others. It is also considered to be an evidence of sewage pollution, but this is not at all certain since it occurs in cultivated soils (Jordan, Bacteriology, 1908, page 321).

CHAPTER XXXIV.

THE CHOLERA SPIRILLUM (SPIRILLUM CHOLERÆ ASIATICÆ) AND ALLIED VARIETIES.

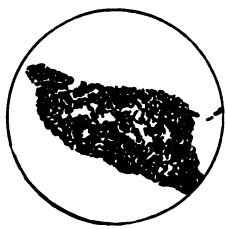
In 1883 Koch separated a characteristically curved organism from the dejecta and intestines of cholera patients—the so-called "*comma bacillus.*" This he declared to be absent from the stools and intestinal contents of healthy persons and of persons suffering from other affections. The organism was said to possess certain morphological and biological features which readily distinguished it from all previously described organisms. It was absent from the blood and viscera, and was found only in the intestines; and the greater the number, it was said, the more acute the attack. Koch also demonstrated an invasion of the mucosa and its glands. The organisms were found in the stools on staining the mucous flakes or the fluid with methylene blue or fuchsin, and sometimes alone; by means of cultivation on gelatin they were readily separated from the stools. Numerous control observations made upon other diarrhœic dejecta and upon normal stools were negative; the comma bacillus was found in choleraic material only, or occasionally in small numbers in the stools of healthy persons who came in contact with cholera. Soon, however, other observers described comma-shaped organisms of non-choleraic origin. Finkler and Prior, for instance, found them in the diarrhœal stools of cholera nostras, Deneke in cheese, Lewis and Miller in saliva. All of these organisms, however, differed in many respects from Koch's comma bacillus, and it has since been proved that none of them is affected by the specific serum of animals immunized to cholera. After a time, therefore, the exclusive association of Koch's vibrio with cholera or those in contact with it became almost generally acknowledged, until now it is regarded by bacteriologists everywhere to be the specific cause of Asiatic cholera. Certain sporadic cases of cholera-like disease, however, are undoubtedly due to other organisms.

Morphology.—Curved rods with rounded ends which do not lie in the same plane, of an average of 3 to 5μ in length and about 0.4μ in breadth. The curvature of the rods may be very slight, like that of a comma, or distinctly marked, particularly in fresh unstained preparations of full-grown individuals, presenting the appearance of a half-circle. By the inverse junction of two vibrios S-shaped forms are produced. Longer forms are rarely seen in the intestinal discharges or from the cultures grown on solid media, but in fluids, especially when grown under unfavorable conditions, long, spiral filaments may develop. The spiral forms are best studied in the hang-

ing drop, for in the dried and stained preparations the spiral character of the long filaments is often obliterated. In film preparations from the intestinal contents in typical cases it will be found that the organisms are present in enormous numbers, and often in almost pure culture (Figs. 142 and 143). In old cultures irregularly clubbed and thickened involution forms are frequent, and the presence in the organisms of small, rounded, highly refractile bodies is often noted.

Staining.—The cholera spirillum *stains* with the aniline colors usually employed, but not as readily as many other bacteria; a dilute aqueous solution of carbol fuchsin (1.0 per cent.) is recommended as

FIG. 142



Contact smear of colony of cholera spirilla rom agar. × 700 diameters. (Dunham.)

FIG. 143

Cholera spirilla preparation from gelatin-plate culture of cholera. × 800 diameters.

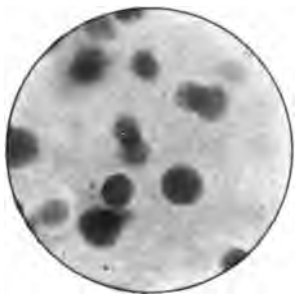
the most reliable staining agent with the application of a few minutes' heat. It is decolorized by Gram's method. The organisms exhibit one long, fine, spiral flagellum attached to one end of the rods, or, exceptionally, to both ends. (Cholera-like spirilla often have 1, 2, or 3 end flagella.) In sections they are stained best by alkaline methylene-blue solution and washed in water slightly acidulated with acetic acid.

Biology.—An aërobic (facultative anaërobic), liquefying, very motile spirillum. Grows readily in the ordinary culture media, best at 37° C., but also at room temperature (22° C.); does not grow at a temperature above 42° or below 8° C., and does not form spores.

In *gelatin-plate cultures* at 22° C. the colonies are quite characteristic; at the end of twenty-four hours, small, round, yellowish-white to yellow colonies may be seen in the depths of the gelatin, which later grow toward the surface and cause liquefaction of the medium, the colonies lying at the bottom of the holes or pocket thus formed. The zone of liquefaction, which increases rapidly, at first remains clear, then becomes cloudy, mostly gray, as the result of the growth of the colonies. In many cases after a time concentric rings, increasing from day to day, appear in the zone of liquefaction. (See Figs. 144 and 145.) Examined under a low-power lens, at the end of sixteen to twenty-four hours, the colonies appear as small, light

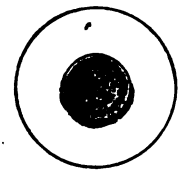
yellow, round, coarsely granular disks, with a more or less irregular outline. In many cases at this stage an ill-defined halo is seen to surround the granular colony. As the colonies become older the granular structure increases, until a stage is reached when the surface looks as if it were covered with little fragments of broken glass. Liquefaction continues about the colonies, their structure appears fissured and coarsely granular in texture, and occasionally a hair-like border is formed at the periphery (Fig. 145). Sometimes the colonies may be retained as compact masses in the zone of liquefaction, and then they are dark yellow or brown in color, and forms occur which are

FIG. 144



Cholera colonies in gelatin; twenty-four to thirty-six hours' growth. × about 20 diameters.

FIG. 145



Cholera colony in gelatin, liquifaction beginning. × 30 diameters. (Dunham.)

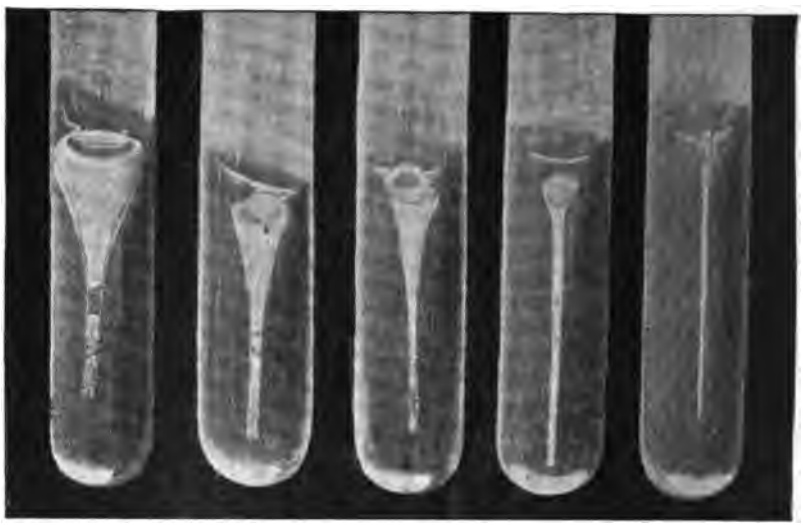
absolutely unlike the typical cholera colonies. In *gelatin-stick cultures* the growth is at first thread-like and uncharacteristic. At the end of twenty-four to thirty-six hours a small, funnel-shaped depression appears on the surface of the gelatin, which soon spreads out in the form of an air bubble above, while below this is a whitish, viscid mass. Later, the funnel increases in depth and diameter, and at the end of from four to six days may reach the edge of the test-tube; in from eight to fourteen days the upper two-thirds of the gelatin is completely liquefied. (See Fig. 146.) Freshly isolated cholera vibrios liquefy gelatin more rapidly than old laboratory cultures; a certain variation, under some circumstances, in the characteristic liquefaction on the gelatin, even in fresh cultures, should be borne in mind in making a diagnosis. Such variations in cultural peculiarities occur also with other bacteria.

Upon the *surface of agar* the *Comma bacillus* develops a moist, shining, grayish-yellow layer. In *agar-plate cultures*, for diagnostic purposes, the growth of separated colonies is of some importance.

The nutrient agar after pouring in the plates and solidifying should be slightly dried on the surface by putting the uncovered plate face downward on the shelf of the incubator at 37° C. for thirty minutes, or at 60° C. for five minutes. The cholera colonies develop fairly characteristically, being more transparent than those of most other bacteria except the cholera-like vibrios. *Blood serum* is rapidly

liquefied at the temperature of the incubator. On *potato* at incubator temperature a moist growth of a dirty brown color occurs. *Milk* is not coagulated. In *bouillon* the growth is rapid and abundant; in the incubator at the end of ten to sixteen hours the liquid is diffusely colored, and on the surface a wrinkled membranous layer is often formed. In general the spirillum grows in any liquid containing a small quantity of organic matter and having a slightly alkaline reaction. An acid reaction of the culture medium prevents its development, as a rule; but it has the power of gradually accommodating itself to

FIG. 146



A characteristic series of cholera cultures in gelatin; from right to left, one, two, three, four, and six days' growth. (Dunham.)

the presence of vegetable acids. Abundant development occurs in bouillon which has been diluted with eight to ten parts of water and in simple peptone solution.

The comma bacillus belongs to the class of aërobic organisms, inasmuch as it grows readily only in the presence of oxygen, and that it develops active motility only when a certain amount of oxygen is present. It does not grow in the total absence of oxygen, but a small quantity of oxygen is all that is required for its development, as in the intestines. This need of oxygen tends to send the spirilla to the surface of fluid culture media.

Cholera-red Reaction.—When a small quantity of chemically pure sulphuric acid is added to a twenty-four-hour bouillon culture of the cholera bacillus containing peptone a reddish-violet color is produced. Brieger separated the pigment formed in this reaction—the so-called *cholera-red*—and showed that it was indol, and that the reaction was nothing more than the well-known indol reaction. Salkowski and

Petri then demonstrated that the cholera bacilli produced in thin bouillon cultures, along with indol, nitrites by reducing the nitrates contained in small quantities in the culture media. They showed that it is the nitric acid, liberated by the addition of sulphuric acid to the culture, which would give rise to the indol, the red body upon which the cholera reaction depends. For a long time it was believed that this nitroso-indol reaction was peculiar to the cholera bacillus and great weight was placed on it as a diagnostic test. It has since been shown, however, that there are a number of other vibrios which, under similar conditions as the cholera vibrio, give the same red reaction. The reaction is, nevertheless, a constant and characteristic peculiarity of this spirillum and is of unquestionable value. It is even more valuable as a negative than as a positive test, as the absence of the reaction enables one to say of a suspected organism that it is not the cholera spirillum. There are, however, certain precautions to be observed in its use. It has been shown that the reaction may be absent, for instance, when the culture contains either too much or too little nitrate. It is, therefore, advisable not to employ a bouillon culture the composition of which is uncertain, but a distinctly alkaline solution of peptone, containing 1 per cent. pure peptone and 0.5 per cent. of pure chloride of sodium (Dunham's solution). With such a solution constant results can be obtained.

Development Outside of the Body.—It has been shown by experiment that cholera spirilla multiply to some extent in sterilized river-water or well-water, and preserve their vitality in such water for several weeks or even months. Koch demonstrated the presence of this spirillum in the foul water of a tank in India which was used by the natives for drinking purposes. In his early investigations he found that rapid multiplication may occur upon the surface of moist linen.

Resistance and Vitality.—If a culture be spread on a cover-glass and exposed to the action of the air at room temperature the bacilli will be dead at the end of two or three hours, unless the layer of culture is very thick, in which case it may take twenty-four hours or more to kill all the bacilli. This indicates that infection is not produced by means of dust or other dried objects contaminated with cholera bacilli. The transmission of these organisms through the air, therefore, can only take place for short distances, as by a spray of infectious liquids by mechanical means—as, for instance, the breaking of waves in a harbor, on water-wheels, etc., or in moist wash of cholera patients.

The cholera bacillus is also injuriously affected by the abundant growth of saprophytic bacteria. It is true that when associated with other bacteria, if present in large numbers, and if the conditions for their development are particularly favorable, the cholera bacillus may at first gain the upper hand, as in the moist linen of cholera patients, or in soil impregnated with cholera dejecta; but later, after two or three days, even in such cases, the bacilli die off and other

bacteria gradually take their place. Thus, Koch found that the fluid contents of privies twenty-four hours after the introduction of comman bacilli no longer contained the living organisms; in impure river-water they were not demonstrable for more than six to seven days, as a rule. In the dejecta of cholera patients they were found usually only for a few days (one to three days), though rarely they have been observed for twenty to thirty days, and on one occasion for one hundred and twenty days. In unsterilized water they may also retain their vitality for a relatively long time; thus, in stagnant well-water they have been found for eighteen days, and in an aquarium containing plants and fishes, the water of which was inoculated with cholera germs, they were isolated several months later from the mud at the bottom. In running river-water, however, they have not been observed for over six to eight days. For the cholera organisms the conditions favorable to growth are a warm temperature, moisture, a good supply of oxygen, and a considerable proportion of organic material. These conditions are fully met with outside the body in but very few localities.

The comma bacillus has the average resistance of spore-free bacteria, and is killed by exposure to moist heat at 60° C. in ten minutes, at 95° to 100° C. in one minute. The bacilli have been found alive kept for a few days in ice, but ice which has been preserved for several weeks does not contain living bacilli.

Chemical disinfectants readily destroy the vitality of cholera vibrios. For disinfection on a small scale, as for washing the hands when contaminated with cholera infection, a 0.1 per cent. solution of bichloride of mercury, or a 2 to 3 per cent. solution of carbolic acid may be used. For disinfection on a large scale, as for the disinfection of cholera stools, strongly alkaline milk of lime is an excellent agent. The wash of cholera patients, contaminated furniture, floors, etc., may be disinfected by a solution of 5 per cent. carbolic acid and soap water.

Pathogenesis.—Not one of the lower animals is naturally subject to cholera, nor has any contracted the disease as the result of the ingestion of food contaminated with choleraic excreta or from the inoculations of pure cultures of the spirillum, either subcutaneously or by the mouth. It has been shown that the comma bacillus is extremely sensitive to the action of acids, and is quickly destroyed by the acid secretions of the stomach of man or the lower animals, when these secretions are normally produced. Koch sought to produce infection in guinea-pigs *per vias naturales* by first neutralizing the contents of the stomach with a solution of carbonate of soda—5 c.c. of a 5 per cent. solution injected into the stomach through a pharyngeal catheter—and then after a while administered through a similar catheter 10 c.c. of a liquid into which had been put one or two drops of a bouillon culture of the comma bacillus. The animal then receives a dose of 1 c.c. of tincture of opium per 200 grams of body-weight, introduced into the abdominal cavity, for the purpose

of controlling the peristaltic movements. As a result of this treatment the animals are completely narcotized for about half an hour, but recover from it without showing any ill effects. On the evening of the same or the following day the animal shows an indisposition to eat and other signs of weakness, its posterior extremities become weak and apparently paralyzed, and, as a rule, death occurs within forty-eight hours with the symptoms of collapse and fall of temperature. At the autopsy the small intestine is found to be congested and filled with a watery fluid, containing the spirillum in great numbers. These results, however, are somewhat weakened by the fact that experiments made with some other bacteria morphologically similar to the comma bacillus of Koch, but specifically different, occasionally produced death when introduced in the same way into the small intestines of guinea-pigs.

There are several cases on record which furnish the most satisfactory evidence that the cholera spirillum is able to produce the disease in man. In 1884 a student in Koch's laboratory in Berlin, who was taking a course on cholera, became ill with a severe attack of cholera. At that time there was no cholera in Germany, and the infection could not have been produced in any other way than through the cholera cultures which were being used for the instruction of students. In 1892 Pettenkofer and Emmerich experimented on themselves by swallowing small quantities of fresh cholera cultures obtained from Hamburg. Pettenkofer was affected with a mild attack of cholerine or severe diarrhœa, from which he recovered in a few days without any serious effects, but Emmerich became very ill. On the night following the infection he was attacked by frequent evacuations of the characteristic rice-water type, cramps, tympanites, and great prostration. His voice became hoarse, and the secretion of urine was somewhat diminished, this condition lasting for several days. In both cases the cholera spirillum was obtained in pure culture from the dejecta. Finally, there is the case of Dr. Oergel, of Hamburg, who accidentally, while experimenting on a guinea-pig, allowed some of the infected peritoneal fluid to squirt into his mouth. He was taken ill and died a few days afterward of typical cholera, though at the time of his death there was no cholera in the city. These accidents and experiments would certainly seem to prove conclusively the capability of pure virulent cholera cultures to produce the disease.

Lesions in Man.—Cholera in man is an infective process of the epithelium of the intestine, in which the spirilla clinging to and between the epithelial cells produce a partial or entire necrosis and final destruction of the epithelial covering, which thus renders possible the absorption of the cholera toxin formed by the growth of the spirilla. The larger the surface of the mucous membrane infected and the more luxuriant the development of bacilli and the production of toxin, the more pronounced will be the poisoning, ending fatally in a toxic paralysis of the circulatory and thermic centres.

On the other hand, however, there may be cases where, in spite of the large number of cholera bacilli present in the dejecta, severe symptoms of intoxication may be absent. In such cases the destruction of epithelium is not produced or is so slight that the toxic substance absorbed is not in sufficient concentration to give rise to the algid stage of the disease, or for some reason the spirilla do not produce toxin to any extent. In no stage of the disease are living cholera spirilla found in the organs of the body or in the secretions.

Distribution in the Body.—The cholera spirilla are found only in the intestines and are believed never to be present in the blood or internal organs. The lower half of the small intestine is most affected, a large part of its surface epithelium becoming shed. The flakes floating in the rice-water discharges consist mostly of masses of epithelial cells and mucus, among which are numerous spirilla. The spirilla also penetrate the follicles of Lieberkühn, and may be seen lying between the basement membrane and the epithelial lining, which become loosened by their action. They are rarely found in the connective tissue beneath, and never penetrate deeply. In more chronic cases other microörganisms play a greater part and deeper lesions of the intestines may occur.

Communicability. Origin of Epidemics.—From this fact and other known properties of the cholera spirillum, which have already been referred to, several important deductions may be made with regard to the mode of transmission of cholera infection. In the first place, the bacilli evidently leave the bodies of cholera patients, chiefly in the dejections during the early part of the disease (they have usually disappeared after the fourth to the fourteenth day, but may remain for many months), and only these dejections, therefore, and objects contaminated by them, such as bed and body linen, floors, vaults, soil, well-water and river-water, green vegetables wet with infected water, etc., can be regarded as possible sources of infection. There is a special limitation even in these sources of infection, owing to the fact that this spirillum is so easily destroyed by desiccation and crowded out by saprophytic organisms. Thus, as a rule, only fresh dejections and freshly contaminated objects are liable to convey infection; a day after they have become completely dry there is little danger. Further, we must conclude from the distribution of the cholera bacillus in the body and from experiments upon animals that the commonest mode of infection is by way of the mouth, and chiefly by means of water used for drinking purposes, for the preparation of food, etc. In recent times cholera spirilla have been found not infrequently in water (wells, water-mains, rivers, harbors, and canals) which has become contaminated by the dejections of cholera patients.

As in other infectious diseases, not everyone who is exposed to infection is attacked by cholera. The bacilli have been found during cholera epidemics in the dejections of healthy individuals without any pathological symptoms. Abel and Claussen, for example,

in 14 out of 17 persons belonging to the families of 7 cholera patients, found cholera vibrios, in some of them for a period of fourteen days. In Hamburg there were 28 such cases of healthy choleraic individuals with absolutely normal stools. It is evident, therefore, that an individual susceptibility is requisite to produce the disease. In the normal healthy stomach the hydrochloric acid of the gastric secretions may destroy the spirilla; and, finally, the normal vital resistance of the tissue cells to the action of the cholera poison may be taken into consideration. According to the greater or less power of this vital resistance of the body the same infectious matter may give rise to no disturbance whatever, a slight diarrhœa, or it may lead to serious results. Furthermore, it may be accepted as an established fact that recovery from one attack of cholera produces personal immunity to a second attack for a considerable length of time. This does not appear to depend upon the severity of the attack, for cases are recorded of persons who were apparently not sick at all and yet in whom an acquired immunity was produced. How long this immunity lasts is not positively known, but probably for a month or more, so that the same person is not likely to be taken ill again with cholera during an epidemic.

On the other hand, we may take it for granted that susceptibility to cholera may be acquired or increased. For instance, there is no doubt that gastric and intestinal disorders produced by overheating, etc., may act as contributing causes to the disease. Other predisposing causes are general debility from poverty, hunger, disease, etc.

Flies which have fed or lighted on the discharges of cholera patients or on things contaminated by them have been found to carry the organisms not only on their feet, but also in their bodies for at least twenty-four hours. Food contaminated by flies is therefore a possible souce of infection. Even vegetables such as lettuce may be contaminated by infected water. Cholera, as a rule, starts from Asia, travels to Europe, and is carried by vessels to America.

Cholera Toxins.—Koch was the first to assume, as the result of his investigations, that the severe symptoms of the algid stage of cholera were due to the effects of a toxin produced by the growth of the comma bacillus in the intestines.

In 1892 Pfeiffer published an account of his elaborate researches relating to the cholera poison. He found that recent aërobic cultures of the cholera spirillum contain a specific toxic substance which is fatal to guinea-pigs in extremely small doses. There is extreme collapse, with subnormal temperature. This substance stands in close relation with the bacterial cells, and is perhaps an integral part of them. The filtrate of a recent cholera culture contains usually only moderate amounts of toxic substances. The spirilla may be killed by chloroform, thymol, or by desiccation, without apparent injury to the toxic power of this substance, but subjected to 60° C. some of the toxins are destroyed. Metchnikoff, Roux, and others have shown that living, highly virulent cultures produce at times highly poisonous

toxins, the 0.2 c.c. of filtrate of a three- to four-day culture killing 100 grams of guinea-pig. The living culture in 2 to 4 c.c. of nutrient bouillon contained in collodion sacs, when placed in the peritoneal cavity of guinea-pigs, produced symptoms of poisoning and death in a few days. Sacs containing the dead vibrios produced little effect. There appears to be, therefore, considerable difference between the intracellular and the soluble extracellular toxins.

Cholera Immunity and Bacteriolysins.—Koch found in his animal experiments that recovery from an intraperitoneal infection with small doses of living cholera vibrios produced a certain immunity against larger doses, though the animals inoculated were not very much more resistant to the cholera poison than they were originally. In 1892 Lazarus observed that the blood serum of persons who had recently recovered from an attack of cholera possessed the power of preventing the development in guinea-pigs of cholera bacilli, which in these animals are rapidly fatal when injected intraperitoneally, while the serum of healthy individuals had no such effect. This specific change in the blood is observed to take place from eight to ten days after the termination of an attack of cholera, and reaches its maximum during the fourth week of convalescence, after which it declines rapidly and disappears entirely in about two or three months. Similar antitoxic or bactericidal substances develop in the serum of guinea-pigs, rabbits, and goats, when these animals are immunized artificially against cholera by subcutaneous or intraperitoneal injections of living or dead cultures. These specific substances present in the blood of cholera-immune men and animals act only upon organisms similar to those with which they were infected; but, as Pfeiffer showed, this specific relation, which is found to exist between the antibacterial and protective substances produced during immunization and the bacteria employed to immunize the animals, is not confined to cholera. The discovery, moreover, of this specific reaction of the blood serum of immunized man and animals when brought in contact with the spirilla, has given us an apparently reliable means of distinguishing the cholera from all other vibrios, and the disease cholera from other similar affections, both of which have proved to be of great value, particularly in obscure or doubtful cases, in which heretofore the only method of differential diagnosis available—viz., by cultural tests—was often unsatisfactory.

Anticholera Inoculations.—Within the last ten years Haffkine, in India, has succeeded in producing an artificial immunity against cholera infection by means of subcutaneous injections of cholera cultures. Two or three injections are necessary to give the greatest amount of protection. Animals treated by this method are refractory to intraperitoneal inoculations, but not to intestinal injections, when fed by Koch's method. In the intestines the bacteria seem to be outside the influence of the bactericidal properties of the blood, and the absorption of toxins is too great to be neutralized by the small amount of antitoxin. In over 200,000 persons inoculated under his super-

vision the results obtained would seem to show a distinct protective influence in the preventive inoculations. Great care must be taken that the vaccine is sterile. Through lack of care a number of cases of tetanus were caused by a contaminated vaccine. The injections produce local swelling and a short rise of temperature with possibly headache.

Serum Therapy.—Up to the present no successful results have been reported. The outlook is also not very hopeful. A bactericidal serum can be developed, but this has not saved animals showing toxic symptoms.

Agglutinins.—Five to ten days after infection (natural or experimental) agglutinins appear in the blood of man or animal. These are at least in part specific. Their presence in the blood is of diagnostic importance. When present in great amount, such agglutinins can be used for identifying doubtful spirilla. In their agglutination with a specific serum they are also alike. Some cultures agglutinate with more difficulty than others, so that the same serum may agglutinate different cultures in dilutions varying from 1: 1000 up to 1: 10,000. Such a serum would not agglutinate cholera-like spirilla above a 1 : 50 dilution. Epecially among isolated cases of cholera-like diseases spirilla are met with which do not agree in agglutination characteristics.

Variations of the Cholera Spirillum.—From the great majority of all cases of epidemic cholera examined, cholera spirilla agreeing in all essential characteristics have been obtained, usually in great numbers and often in almost pure culture.

Biological Diagnosis of the Cholera Vibrio. Plan of Procedure.—*A.* Dejecta (fluid) or intestinal contents of a cholera patient or cholera suspect.

1. Use one drop (one platinum loop) for gelatin-plate cultures, making two dilutions. Do this in duplicate or triplicate. Cultivate at 22° C.

2. Inoculate a couple of bouillon tubes and a couple of Dunham's 1 per cent. peptone solution with one drop each, and place them in the incubator (37° to 38° C.) for six to eight hours.

3. Examine a drop of the dejecta in a hanging drop.

4. Examine a drop of the dejecta in stained cover-glass preparation.[1]

5. Make gelatin plates from one drop taken from the surface of each of the bouillon and peptone solution tubes and cultivate at 22° C.

[1] These direct microscopic examinations of the intestinal contents are, as a rule, very unsatisfactory, at least in those in which the symptoms are not marked. In a few the spirals will make up from 50 to 100 per cent. of the bacteria present. In most of the cases during the last epidemic in New York Dunham found abundance of columnar epithelium from the intestinal mucous membrane, numerous straight, thick bacilli, and only a few curved bacilli or segments of spirals—too few to identify. Plate cultures from these showed from 20 to 80 per cent. of all the colonies developing to be cholera spirilla.

6. As soon as the plates (see 1 and 5) are sufficiently developed (thirty-six to forty-eight hours) fish the suspected cholera colonies and use the material for the following procedures:

7. Inoculate six or eight peptone tubes (1 per cent. peptone and 0.5 per cent. NaCl in distilled water) and place them at once in the incubator. Note the time.

8. Examine hanging drop for form, size, and motility (and arrangement).

9. Make stained cover-glass preparations and examine.

10. Then try indol reaction with the same tubes.

11. While these tubes are incubating use material from the suspected colonies on the plates (1 and 5) for hanging-drop cultures.

12. Meanwhile make stained cover-glass preparations from other colonies of suspected cholera on the plates (1 and 5).

13. Make gelatin-tube cultures from colonies on plates (1 and 5) and study cultures daily for five or six days, to observe the shape of growth along the line of puncture.

B. Suspected water.

Add to 500 c.c. or 1 litre of the water to be examined enough peptone-salt solution (20 per cent. peptone and 10 per cent. NaCl) to make a 1 per cent. solution of peptone. Then proceed as in *A*.

Specific Serum Reactions.—All authors now agree that the differentiation of the cholera vibrio from other similar vibrios cannot always be made by the cultural method, nor is the usual inoculation of animals sufficient. For this purpose serum is employed either by making intraperitoneal injections of a surely fatal dose of the suspected spirillum along with the serum of animals immunized to undoubted cholera cultures, so as to note whether specific protection is afforded, or the agglutination test is carried out in such a way as to determine if specific agglutination of the spirilla occurs.

SPIRILLA MORE OR LESS ALLIED TO THE CHOLERA SPIRILLUM.

The examinations of the stools of persons suffering from cholera have revealed, in a small percentage of cases, spirilla resembling either very closely or having a fair degree of similarity to the true cholera organisms. Further, in a small percentage of cases having choleraic symptoms no true cholera vibrios have been found, but instead other spirilla resembling them more or less closely.

These may differ in having two or more end flagella, in size, in production of nitrites, etc., or they may be identical in the tests commonly employed. They all differ in the specific agglutination and bacteriolytic tests from the cholera spirilla and among themselves.

In a recent epidemic in Egypt, Gottschlich obtained from sixteen [illegible]illa differing from the true spirilla, and found every one [illegible] some characteristic from all others. Some were patho- [illegible] pigeons through inoculation of a small quantity into the [illegible]uscle; others were atypical in their development in nutrient

gelatin. None of these microörganisms injected into animals induced production of agglutinins for the true cholera spirilla.

Kolle and Gottschlich consider these various spirilla found by them in Egypt as well as others found by different investigators in India, Germany, and elsewhere to be saprophytes. It is more probable, in the writer's opinion, that some of them must be considered as bearing a part in exciting a cholera-like disease, but that they are not very pathogenic and require very favorable conditions, probably long-continued, before they can exert their action.

Some special varieties of spirilla resembling those of cholera have received especial attention on account of having been obtained before it was known that so many cholera-like vibrios existed. The vibrio Berolinensis, cultivated by Neisser from Berlin sewage-water; the vibrio Danubicus, cultivated by Hauser from canal-water, and the vibrio of Massowah, cultivated by Pasquale from a case during an epidemic of cholera, all are negative to the specific serum reactions, and differ in the number of terminal flagella or in other characteristics. Cunningham found a number of such spirilla in cases of apparently true cholera in India. Some of these may have been true cholera spirilla and others may have had some relationship to the disease in the person from which they were derived.

FIG. 147

Spirillum of Finkler and Prior. × 1100 diameters.

Spirillum of Finkler and Prior.—Because of their prominence in literature and their frequent use in teaching, the spirillum of Finkler and Prior, that of Metchnikoff, and that of Deneke are of considerable interest.

Finkler and Prior, in 1884, obtained from the fæces of patients with cholera nostras, after allowing the dejecta to stand for some days, a spirillum which is of interest mainly because it simulates the comma bacillus of Koch, but differs from it in several cultural peculiarities.

Morphology.—Somewhat longer and thicker than the spirillum of Asiatic cholera and not so uniform in diameter, the central portion being usually wider than the pointed ends.

Biology.—An aërobic and facultative anaërobic, liquefying spirillum. Does not form spores. Upon *gelatin plates* small, white, punctiform colonies are developed at the end of twenty-four hours. These are round, but less coarsely granular, darker in color, and with a more sharply defined border than the comma bacillus. Liquefaction of the gelatin around these colonies progresses rapidly, and at the end of forty-eight hours is usually complete in plates where they are numer-

ous. In *gelatin-stick cultures* liquefaction progresses much more rapidly than in similar cultures of the cholera spirillum, and a stocking-shaped pouch of liquefied gelatin, already seen after forty-eight hours, is filled with a cloudy liquid. The liquefaction increases, and in twenty-four hours more reaches the sides of the tube in the upper part of the medium; by the end of the week the gelatin is usually completely liquefied. Upon the surface of the liquefied medium a whitish film is seen. Upon *agar* there is a somewhat more luxuriant growth than is seen with the cholera vibrio. Upon *potato* this spirillum grows at room temperature and produces a slimy, grayish-yellow, glistening layer which soon extends over the entire surface. The cholera spirillum does not grow at room temperature, and in the incubator produces a thin, brownish layer. The absence of agglutination with a suitable dilution of the serum of an animal immunized to the cholera spirillum is a valuable differential sign.

In 1884 Miller observed a curved bacillus in a hollow tooth, which from its behavior in microscopic preparations, in cultures, and animal experiments, is probably identical with the Finkler and Prior spirillum. Very similar spirilla have been found by others.

Spirillum of Metchnikoff.—Discovered in 1888, in Odessa, by Gamaleïa in the intestinal contents of fowls dying of an infectious disease, which prevails in certain parts of Russia during the summer months, and which presents symptoms resembling fowl cholera. Gamaleïa's experiments show that this organism is the cause of the disease mentioned. It has since been found by Pfuhl and Pfeiffer in the water of the Spree at Berlin and in the Lahn by Kutchler.

Morphology.—Morphologically this spirillum is almost identical with the cholera spirillum. In the blood of inoculated pigeons the diameter is sometimes twice as great as that of the cholera spirillum, and almost coccus-like forms are often found. A single, long, undulating flagellum is attached to one end of the spiral filaments or curved rods.

Stains with the usual aniline colors, but not by Gram's method.

Cultural Characters.—Upon *gelatin plates* the vibrio Metchnikoff grows considerably faster than the cholera vibrio; small, white, punctiform colonies are developed at the end of twelve hours; these rapidly increase in size and cause liquefaction of the gelatin within twenty-four to thirty hours. At the end of three days large, saucer-like areas of liquefaction may be seen, the contents of which are turbid, as a rule. In *gelatin-stick cultures* the growth is almost twice as rapid as the cholera bacillus. In *bouillon* at 37° C. development is very rapid, and the liquid becomes clouded and opaque, and a thin, wrinkled film forms upon the surface. On the addition of the pure sulphuric acid to twenty-four-hour peptone cultures a distinct nitrosoindol reaction is produced. *Milk* is coagulated and acquires a strongly acid reaction. The spirillum is not agglutinated by the specific cholera agglutinin.

Pathogenesis.—The vibria of Metchnikoff is pathogenic for fowls, pigeons, and guinea-pigs. A small quantity of a virulent culture

fed to chickens and pigeons causes their death with the local and general symptoms of fowl cholera. At the autopsy the most constant appearance is hyperæmia of the entire alimentary canal. A grayish-yellow liquid, more or less mixed with blood, is found in considerable quantity in the small intestine. In the watery fluid large numbers of spirilla are found. A few drops of a pure culture inoculated subcutaneously in pigeons produce septicæmia and cause their death in twelve to twenty-four hours.

In contradistinction to the pathogenic virulence of these spirilla for pigeons and guinea-pigs, the cholera spirillum is much less pathogenic. Pigeons are not killed by the intramuscular inoculation of pure fresh cultures of the vibrio choleræ. The pathogenic action of the vibrio Metchnikoff upon pigeons and guinea-pigs, producing in these animals general septicæmia and death, is, therefore, a characteristic point of difference between this and the spirillum of Asiatic cholera.

Within recent years numerous other somewhat similar spirilla, the so-called "water vibrios," have been found while looking for the cholera spirillum.

CHAPTER XXXV.

PATHOGENIC MICRO-ORGANISMS BELONGING TO THE HIGHER BACTERIA (TRICHOMYCETES).

THE members of the higher bacteria which are pathogenic for man have as yet been incompletely studied and classified. The following divisions serve as an attempt at differentiation:

1. *Leptothrix* grows in stiff, almost straight threads, in which division processes are seldom or never observed, and no branching has been seen.

2. *Cladothrix* grows in threads which rapidly fragment and produce false branching, that is, the terminal cell remains partly attached, but is pushed to one side by further growth from the parent thread, thus a Y-shaped growth is produced, and then bacillary characteristics in old cultures.

3. *Actinomyces* grows in threads with true branching. No spores have been observed. It is characterized by the radiating wreath-like forms which it alone produces in the living body.

4. *Nocardia* (*Streptothrix*) grows in threads which produce abundant true branching, later there is fragmentation, and formation of conidia, which serve as organs of propagation, and in this sense may be considered as spores.

Foulerton considers all organisms in the group classed as higher bacteria as belonging to a single genus, streptothrix, which he places with the hyphomycetes, or mold fungi, because of their growth in branching threads from spore-like bodies. He says that streptothrix and actinomyces are absolutely synonymous terms, and that the majority of pathologists consider them so.

It seems to us, however, that more minute work, both clinical and experimental, should be done on this group of infections before this classification can be accepted. The term streptothrix, too, is misapplied, since it was used in 1839 for a mold (see p. 465).

These higher bacteria may rightly be considered, according to their development, as a transition group between the simple bacteria and the more highly developed fungi.

The *nocardia* group of microörganisms while having many affinities with the bacteria seem to be more closely related to the true moulds than any of the others. They develop from spore-like bodies into cylindrical dichotomously branching threads, which grow into colonies the appearance of which suggests a mass of radiating filaments. Under favorable conditions certain of the threads become fruit hyphæ, and these break up into chains of round, spore-like bodies, which do not, however, have the same staining reactions nor resisting powers as bacterial spores. The tubercle, grass and diphtheria bacilli are by

some believed properly to belong in this group, on account of the apparent branching forms developed by them under certain conditions, but if not classed with the true bacteria, they should either be put in a group by themselves or be classed with the cladothrix group since their apparent branching takes place in a manner similar to that described as occurring in the latter group.

Foulerton and his associates have made an extensive study of this group of microorganisms both saprophytic and parasitic (see bibliography), and they call attention to the acid-fast character of some of the varieties and of the apparent relationship of the group to *B. tuberculosis*, *B. mallei*, and *B. diphtheriæ*. To us, however, the relationship does not seem to be close enough to place all of these organisms in one group. We have shown (see p. 17) that the apparent branching in *B. diphtheriæ* is not a true branching.

Leptothrix Infections.—Leptothrix forms are frequently found in the human mouth (*Leptothrix buccalis*), and one or two writers have claimed that under certain conditions these may become pathogenic, but since no corroborative work has been done, and very little is known about the group, no opinion can be formed of the worth of these observations.

Cladothrix Infections.—The organisms found in the comparatively few cases which have been considered by their observers to be due to cladothrix have not been minutely enough studied to decide definitely as to their true or false branching, the characteristic chosen to separate them from the nocardia; hence it is difficult to separate the two groups, but an attempt should be made, since the difference said to exist between them is a vital one, from a morphologic standpoint. Clinically, however, according to the reports, the cases cited are very similar to those said to be due to nocardia and to actinomyces.

Gasten found in a case of clinically typical actinomycosis, in which abscess cavities were found along the spinal column, not the usual actinomyces in the yellow, granular pus, but a fine mass of filament. Cultures grew on all the ordinary media, best at incubator temperature, but also at lower temperature on gelatin. The gelatin stick culture, which was especially characteristic, formed on the surface a whitish button; delicate threads stretched out in all directions from the point of inoculation. On agar and potato rumpled, folded films with white deposit on the surface, which contained spores. Animal inoculation gave positive results only in a few cases of intraperitoneal injection of rabbits and guinea-pigs. Purulent nodules were found in the peritoneum. Gasten called the organism *Cladothrix liquefaciens*.

Eppinger found in post-mortem examination of a case of chronic cerebral abscess, which was the result of purulent meningitis, in the pus and abscess walls, etc., a delicate fungoid growth which he succeeded in cultivating on various media. On sugar agar it formed yellow, rumpled colonies which finally developed into a skin. On potato it grew rapidly, but the colonies remained small, at first a

white, granular deposit, which afterward turned red, and on the twentieth day resembled a crystallized almond. It did not grow well on gelatin. In bouillon it formed on the surface a small white granule, which became deeper in the centre as it grew and sank to the bottom as a white deposit. The bouillon remained clear.

Microscopically, the fungus consisted of fine threads without branches which exhibited distinct motility. No flagella were observed. It was judged to be a cladothrix, to which the name "asteroides" was given by the author. It proved to be quite pathogenic for rabbits and guinea-pigs, and produced an infection of pseudo-tuberculosis. Mice were not affected by inoculation.

THE MICRO-ORGANISM OF ACTINOMYCOSIS.

The little clumps produced by this parasite were first seen by Von Langenbeck in 1845 and the organism was later discovered by Bollinger (1877) in the ox. It was given the name of actinomyces, or ray fungus, by the botanist Harz.

The characteristics of the microörganisms, first described minutely by Bostroem (1890) and by Wolf and Israel (1891), differed greatly and have led to confusion. Bostroem's organism grew best aërobically and developed well at room temperature. He noted the intimate relation of the organism with fragments of grain, and this led to the finding of similar microörganisms in the outer world on grains, grasses, etc.

There is no doubt that some suppurative processes have been due to organisms of these characteristics, but they do not seem to excite true actinomycosis.

Wolf and Israel described a microörganism from two human cases, which differs from that described by Bostroem, but agrees with the microörganisms obtained by most of the more recent investigators. It grew best under anaërobic conditions and did not grow at room temperature. Its growth was much less luxuriant than Bostroem's microörganism. On the surface of anaërobic agar slant cultures on the third, fourth, and fifth day numerous minute isolated dew-drop-like colonies appeared, the largest pinhead in size. These gradually became larger and formed ball-like, irregularly rounded elevated nodules varying in size up to that of a millet-seed, exceptionally attaining the size of a lentil or larger. As a rule, the colonies did not become confluent, and an apparently homogeneous layer of growth was seen to be made up of separate nodules if examined with a lens. In some instances the colonies presented a prominent centre with a lobulated margin and appeared as rosettes. A characteristic of the colonies was that they sent into the agar root-like projections. In aërobic agar slant cultures no growth or a slow and very feeble growth was obtained. In stab cultures the growth was sometimes limited to the lower portion of the line of inoculation or was more vigorous there. In bouillon, after three to five days,

growth appeared as small white flakes, partly floating and partly collected at the bottom of the tube. Growth occurred in bouillon under aërobic conditions, but was better under anaërobic conditions. The organisms here grow in branching and interlacing filaments, which later tend to break into segments (see Fig. 148). The microörganism in smear preparations from agar cultures appeared chiefly as short homogeneous, usually straight, but also comma-like or bowed rods, whose length and breadth varied. In many cultures short plump rods predominated, and in others longer, thicker, or thinner individ-

FIG. 148

Smear from bouillon culture of actinomyces. × 1500 diameters. (From Wright.)

uals were more numerous. The ends of the rods often showed oval or ball-like swellings. Swollen clubs were formed irregularly in the presence of blood or serous fluids.

Some twenty guinea-pigs and rabbits were inoculated, most of them in the peritoneal cavity, with pieces of agar culture. Eighteen animals were killed after four to seventeen weeks, and four were still alive seven to nine months after inoculation. Seventeen rabbits and one guinea-pig showed at the autopsy tumor growths mostly in the peritoneal cavity and in one instance in the spleen. In the four animals still living tumors were to be felt in the abdominal wall. The tumors in the peritoneal cavity were millet-seed to plum size, and were situated partly on the abdominal wall and partly on the intestines, the omentum, the mesentery, and in the liver or in adhesions. While the surface of the smaller tumors was always smooth, the surface of the larger tumors showed small hemispherical prominences, giving them the appearance of conglomerates of smaller tumors. On section the larger tumors presented a tough capsule from which anastomosing septa extended inward inclosing cheesy masses. Microscopic examination of the tumors showed in all cases but one the presence of typical actinomyces colonies, in most cases with typical

"clubs." The general histological appearance of the tumors was like that of actinomycotic tissue.

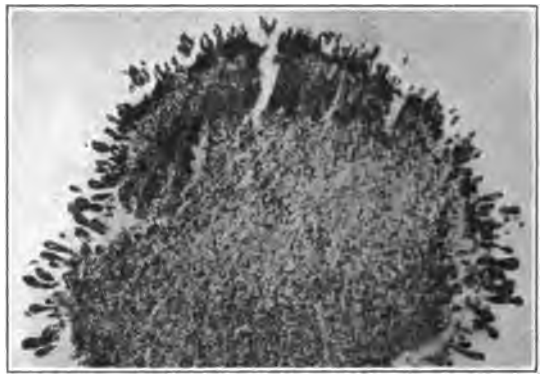
Wolf in a later paper reports that an animal inoculated in the peritoneal cavity with a culture of the same organism had lived a year and a half. At the autopsy several tumors were found in the peritoneal cavity, and in the liver a large typical tumor in which were many colonies which by microscopic examination were shown to be typical club-bearing actinomyces colonies.

Wright in 1905 made an extensive study of actinomycosis and added greatly to our knowledge of it.

Naked-eye Appearance of Colonies of Parasite in Tissues.—In both man and animals they can be readily seen in the pus from the affected regions as small, white yellowish or greenish granules of pin-head size (from 0.5 to 2 mm. in diameter). When pus has not formed they lie embedded in the granulation tissue.

Microscopic Appearance.—Microscopically, these bodies are seen to be made up of threads, which radiate from a centre and present bulbous, club-like terminations (Fig. 149). These club-like terminations are characteristic of the actinomyces. They are generally arranged in pairs, closely crowded together, and are very glistening

FIG. 149



A typical "club"-bearing colony of actinomyces. × 325 diameters. (From Wright.)

in appearance. They are more common in bovine than in human lesions. They have been thought to be reproductive elements, but they are probably simply a reaction of the filament end to the host tissue. The threads which compose the central mass of the granules are from 0.3μ to 0.5μ in diameter: The threads show true branching and in the older colonies show a segmentation which gives them the appearance of chains of cocci. Sometimes the whole centre of the colonies seems to be a mass of cocci, some of which may be true cocci from a mixed infection; the clubs are from 6μ to 8μ in diameter.

The threads are *stained* with the ordinary aniline colors, also by Gram's solution; when stained with gentian violet and by Gram's

method the threads appear more distinct than when stained with methylene blue. The clubs lose their stain by Gram's method and take the contrast strain.

Isolation of Actinomyces.—There are two cultural varieties, one of which grows aërobically and the other anaërobically. The aërobic variety is grown with difficulty. A large number of solidified blood serum or serum agar tubes are inoculated with the hope that one or two will develop a growth. The culture appears much like one of tubercle bacilli. It grows, however, into the medium and takes on a yellowish hue. Wright[1] recommends that granules, preferably obtained from closed lesions, are first thoroughly washed in sterile water or bouillon and then crushed between two sterile glass sides. In bovine cases make sure the granule has filamentous masses, for if not no culture will grow. The crushed granule is transferred to a tube of melted 1 per cent. glucose agar at 40° C. The material is thoroughly distributed by shaking and the tube placed in the incubator. A number of granules after washing should be placed on the inside of a sterile test-tube and allowed to dry. In this way, should the material be contaminated, the drying of the granules for several weeks may kill off the other bacteria. The tube should be examined daily. If a number of living filaments were added to the agar a large number of colonies will develop. These will be most numerous in the depth in a zone five to twelve millimetres below the surface.

Experimental Inoculation in Animals.—True progressive infection is rarely or never obtained by the injection of pure cultures in rabbits, guinea-pigs, or larger animals. It seems as if cultures on artificial media must lose in virulence or that the disease is produced by the entrance of the organism along with some irritating body. When animals are inoculated, the cultures form the characteristic "club"-bearing colonies in the tissues of the experimental animals. These colonies are either enclosed in small nodules of connective tissue or are contained in suppurative foci within nodular tumors made up of connective tissues in varying stages of development. The most extensive lesions show little progressive tendency, and in only a very few instances does multiplication of the microörganism in the body of the inoculated animal take place.

Wright does not accept the prevalent belief, based on the work of Bostroem, Gasperini, and others, that the specific infectious agent of actinomycosis is to be found among certain branching microörganisms, widely disseminated in the outer world, which differ profoundly from actinomyces bovis in having spore-like reproductive elements. He thinks that these forms belong to a separate genus, *Nocardia*, and that those cases of undoubted infection by them should be called nocardiosis and not actinomycosis. The term actinomycosis should be used only for those inflammatory processes the lesions of which contain the characteristic granules or "drüsen." That a *Nocardia* ever forms

[1] Journal of Medical Research, May, 1905.

these characteristic structures in lesions produced by it in man or cattle has not been convincingly shown.

As the actinomycosis microörganism does not grow well on the ordinary culture media and practically not at all at room temperature, it seems very probable that it is a normal inhabitant of the buccal cavity and gastrointestinal tract and does not grow outside the body. Many good observers, however, believe the infection is produced by infected grains upon which the organism has grown as a saprophyte. The explanation of the different results is probably that there are different varieties of this organism.

The cultures are quite resistant to outside influences; dried, they may be kept for a year or more; they are killed by an exposure of five minutes to a temperature of 75° C.

Occurrence.—Actinomycosis is quite prevalent among cattle, in which it occurs endemically; it is more rare among swine and horses. Over one hundred cases have been observed in man. The disease is rarely communicated from one animal to another and no case is known where a direct history of human contagion has been obtained. The cereal grains, which from their nature are capable of penetrating the tissues, have been repeatedly found in centres of actinomycotic infection. This usually occurs in the vicinity of the mouth, where injuries have been accidentally caused. The microörganism may also be introduced by means of carious teeth. Cutaneous infection has been produced by wood splinters, and infection of the lungs by aspiration of fragments of teeth containing the fungus. The presence of the microörganism in cereal grains, which was formerly accepted, is denied by Wright and therefore certainly placed in doubt. The further distribution of the fungus after it is introduced into the tissues is effected partly by its growth and partly by conveyance by means of the lymphatics and leukocytes. Not infrequently a mixed infection with the pyogenic cocci occurs in actinomycosis.

Characteristics of Disease in Man and Animals.—In the earliest stages of its growth the parasite gives rise to a small granulation tumor, not unlike that produced by the tubercle bacillus, which contains, in addition to small round cells, epithelial elements and giant cells. After it reaches a certain size there is great proliferation of the surrounding connective tissue, and the growth may, particularly in the jaw, look like, and was long mistaken for, osteosarcoma. Finally, suppuration occurs, which, according to Israel, may be produced directly by the fungus itself.

The course of the disease is very chronic. Usually the first sign is a point of infiltration about the lower jaw or lower on the neck. This almost painless swelling increases and finally softens in its centre. The necrotic tissue finally forces a passage externally or, passing downward, infects the pleura, lungs, mediastinum, or ribs. As a rule, the disease is not accompanied by fever. In cattle the disease is usually situated in some portion of the head, especially in the jaw, tongue, or tonsils, hence called lumpy jaw, wooden tongue, etc. Primary lung,

intestinal, and skin lesions are not infrequent. These local lesions sometimes scatter and produce a general infection and the udder may be involved.

The experimental production of actinomycosis in animals with material directly from cases has been followed by negative or very unsatisfactory results, as in the case of cultures. When artificially introduced into the tissues the organism is either absorbed or encapsulated. If introduced in large quantities multiple nodules are apparently formed in some cases, which may suggest the production of a general infective process; but on closer inspection of these nodules the thread-like portion of the fungus is found to have disappeared, leaving only the remains of the club-like ends, thus showing that no growth has taken place.

Treatment.—In 1892 Nocard showed that cases in animals might be cured by iodide of potassium, calling attention to the fact that Thomassen had recommended this treatment in 1885. It is given in doses of 1½ to 2½ drams once a day. Salmon and Smith (U. S. Bureau of Animal Industry, Circular No. 96) give directions as to its use.

Mycetoma (Madura Foot).—This is a purulent inflammation of the foot occurring primarily in warm climates. The inflammation is accompanied by much irregular enlargement of the foot. Three varieties of this condition have been described based upon the color of the granules found in the diseased area: (1) white, (2) black, and (3) red. The white variety has been studied by Musgrave and Clegg (1907), who have isolated an organism resembling somewhat actinomyces and somewhat the organism isolated by Wright (1898) from a black variety of the disease which is probably a true mould.

NOCARDIA (STREPTOTHRIX) INFECTIONS.

The most familiar name of this group of microörganisms is streptothrix, but, this name had already been used for another genus; therefore, according to the rules of nomenclature, nocardia, which name was proposed by Trevisan in 1889 for the organism discovered by Nocard in *farcin des bœufs* of cattle should be employed. Wright calls attention to the misuse of the term *streptothrix*, and gives the reasons for the employment of the term *nocardia in its place.*

From widely scattered localities and at long intervals of time reports have been published describing unique cases of disease produced by varieties of microörganisms belonging to the genus nocardia. In some of these cases points of similarity can be recognized in the clinical symptoms and the gross pathologic lesions, while others differ widely in both respects. They have been found in brain abscess, cerebrospinal meningitis, pneumonic areas, and in other pathologic conditions. Eppinger injected cultures into guinea-pigs and rabbits, and observed that they caused typical pseudotuberculosis. Consolidation of portions of both lungs, thickening of the peritoneum, and scattered nodules resembling tubercles were noted

by Flexner in a case of human infection as due to nocardia in which the pathologic picture of the disease resembled so nearly that of tuberculosis in human beings that the two diseases could be separated only by finding the causative microörganism in each case. But in no two cases reported up to the present time have the descriptions of the microörganisms found agreed in all particulars. In some cases no attempt at cultivation was made. In other cases numerous and careful plants on various culture-media failed to develop the specific organism. In the remaining cases in which nocardia was obtained in pure culture the descriptions of the growth characteristics essentially differ. In a review of the literature Tuttle was able to find the reports of only twelve cases in which nocardia was found in sufficient abundance to have been an important, if not the principal, factor in producing disease. These cases were all fatal, and only once was the character of the disease recognized during life. As the clinical symptoms and the lesions in the human subject as well as in the animals experimentally inoculated with nocardia often resemble those of miliary tuberculosis, so that a number of these cases have been reported as pseudotuberculosis, the question is naturally suggested whether such cases of nocardia tuberculosis are not more numerous than the few reported cases would indicate. The almost universal prevalence of genuine tuberculosis and the extreme gravity of the disease have so long occupied the attention and study of the medical profession that much is taken for granted, and in cases in which the symptoms and lesions resemble with some closeness those characteristic of the well-known disease they may easily be set down without question to the account of the tubercle bacillus. The cases of nocardiosis reported which simulated tuberculosis have been fatal, and the lesions for the most part have been widely distributed, but in a number of cases old lesions have been found which suggest that the disease may have been localized for a longer or shorter time, and then, by some accident, may have become rapidly general. In this respect also these cases may resemble tuberculosis. Whether all cases of nocardiosis in the human subject are general and fatal or, as in tuberculosis and actinomycosis, whether there may be cases of localized disease which recover, are questions which have not been decided at the present time. The methods employed to demonstrate the presence of tubercle bacilli render nocardia more or less invisible. Again, unless the observer keeps in mind the possibility of nocardia infection, he may not appreciate the importance of finding slender threads with or without branches, and may consider them accidental bacilli, or varieties of leptothrix or nonpathogenic fungi. As the lungs have appeared to be the seat of the primary infection in most of the cases of human nocardiosis it is very desirable that all cases presenting the physical signs of tuberculosis, in which repeated examinations fail to discover the tubercle bacillus, should be systematically examined for threads. In this way alone can the frequency of the disease be determined. Gram's method of

staining or the Ziehl-Neelson solution decolorized with aniline oil seem to be the most reliable agents for demonstrating these organisms. Varieties of nocardia are widely distributed and are not very infrequently met with, but as yet, with the exceptions mentioned above, very little is known about them.

Tuttle's report of the case of general nocardia infection at the Presbyterian Hospital gives such a good clinical, bacteriologic, and pathologic picture of a case of this infection that a considerable portion of it is repeated here:

Six days before her admission to the hospital her illness began with a severe chill and fever and pain in her left side and back. The following day the pain in the side was worse and breathing was difficult. She began to cough and had some expectoration, but no blood was noticed in the sputa. At irregular intervals she had alternating hot and chilly sensations.

On admision, the patient complained of pain in the left side of the chest, cough, fever, weakness, and prostration. Her temperature was 103° and her pulse and respirations were rapid.

The history of the disease and the physical signs indicated an attack of acute lobar pneumonia, the area of consolidation being small and situated in the lower part of the left upper lobe in front. Frequent and violent coughing, with almost no expectoration, pain in the affected side and in the lumbar region, restlessness and sleeplessness, and involuntary urination were the symptoms noted during the first four days in the hospital. The pneumonic area increased somewhat and extended backward to the posterior axillary line, and the temperature was continuous at 103° to 103.5°. On the fifth day the temperature fell two degrees, and signs of resolution appeared in the consolidated area. The apparent improvement, however, was of short duration. On the sixth day the temperature rose to 104.5°, and continued to rise each day, reaching 107.5° shortly before death, which occurred on the ninth day in the hospital and the fifteenth day of the disease. There were repeated attacks of profuse sweating. On the day before her death three indurated swellings beneath the skin were noticed. One, on the left forearm, about the size of a walnut, apparently contained pus. Two, of smaller size, were situated in the right groin.

Blood cultures from a vein in the arm, taken on the sixth day, remained sterile. The leukocyte count on the seventh day was, 36,000.

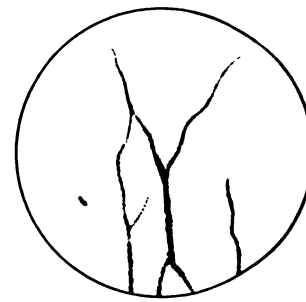
Autopsy.—On the right arm, the left forearm, the abdominal wall, and on both thighs there are eight or ten slightly projecting, rounded, fluctuating, subcutaneous swellings from one-half inch to one inch in diameter. The skin over most of these nodules is unaltered, but over the larger ones there is a slight bluish discoloration. The nodules are composed of bluish-gray, thick, mucilaginous matter, which is very tenacious and can be drawn out into long threads. The lower lobe is thickly studded with miliary tubercles, and scattered

through the entire lung are suppurating foci. Liver and spleen normal. Kidneys: The description of one applies to both. The surface is everywhere and evenly dotted with minute white spots, which suggest septic emboli rather than tubercles. A few prominent

FIG. 150

Streptothrix from bouillon culture. (From Tuttle.)

FIG. 151



Young streptothrix threads showing terminal buds. (From Tuttle.)

white nodules, from one-quarter inch to one-half inch in diameter, contain thick, tenacious matter (Fig. 152). Section shows that the entire substance of the kidney is densely studded with these minute white granules.

The gross pathological conditions were interpreted before nocardia was found as follows: An old tuberculous nodule in the right lung;

FIG. 152

Portion of kidney showing minute and large areas of infection.

acute miliary tuberculosis in the right lung and peritoneum; acute lobar pneumonia, affecting the left lung; septic infarctions and pyæmic abscesses of both lungs, heart muscle, both kidneys, pancreas, mesenteric lymph nodes, and subcutaneous connective tissue. The miliary tuber-

cles of the right lung and peritoneum presented the characteristic appearance of genuine tuberculosis. They were minute, hard, gray, almost translucent nodules, while the granules in the kidneys were of an opaque white or yellowish-white color.

Microscopic Examination.—Smears from the abscesses beneath the skin and on the surface of the kindeys were stained with methyl-blue, carbol-fuchsin, and by Gram's method. The smears resemble those made of tenacious sputum. There is a large amount of mucoid material containing a considerable number of leukocytes. Occasionally irregularly curved, thread-shaped microörganisms are found. They vary considerably in length and thickness, and broken and apparently degenerating fragments are seen. The more slender threads are evenly stained, but some fragmentation or beading of the protoplasm can generally be observed. The thicker threads and broken fragments show deeply stained globules and irregular bodies in a faintly visible rod or thread-shaped covering. Some branching threads are observed, but more commonly they are not branching. No other microörganisms are found in the smears. Sections from the lower lobe of the right lung, stained with hæmatoxylin and eosin, show in certain places the identical microscopic appearances which are considered characteristic of tuberculosis. Stained by Gram's method, with care not to decolorize too completely, threads like those described in the abscesses are found in great abundance, but rather faintly stained. No threads can be found within the typical tubercles with giant cells, but in the zones of small cells around them they are seen in great numbers, winding about among the cells and forming a sort of network. In the minute foci of small cells one or two fragments of threads are generally seen, and a moderate number in the small abscesses. In the areas of more diffuse infiltration these threads are abundant. No other microörganisms can be found except in the pneumonic area of the left lung, where some groups of cocci are seen.

The most reliable staining method and the one requiring the least time is a modified Gram's method. The sections stained with aniline-gentian violet are dipped for a short time in a diluted Gram's iodine solution and then treated with aniline oil until sufficient color has been removed. The aniline oil is then washed out with xylol and the section is mounted in xylol balsam.

Culture Experiments.—Six tubes of Loeffler blood serum were inoculated from the kidneys and kept at 37° C. On the third day minute white colonies appeared in some of the tubes, and on the fifth day all the tubes showed from three to ten or twelve similar colonies in each. The colonies increased in size until some of them reached a diameter of one-eighth of an inch. The color, at first white, changed to yellowish-white and then to a decided pale yellow. The well-developed colonies cling firmly to the surface of the medium and were not easily detached or broken up. The growths in all of the tubes were absolutely pure, and consisted of branching threads like those found in the sections.

Loeffler's blood serum seems to be the most suitable medium for cultures. The growth on this medium is more rapid and abundant than on any of the other media tried.

On plain agar and glycerin agar the growth is the same as on blood serum, but is less rapidly developed.

In bouillon the growth is slow. If the tube is not disturbed or jarred, minute white tufts are seen clinging to the surface of the glass. But if the tube is shaken even slightly they sink slowly to the bottom, forming a white, fluffy layer. These growths when undisturbed resemble minute balls of thistle-down. The yellow color is not apparent even in the mass at the bottom of the tube.

It is strictly aërobic.

Morphology.—When grown on blood serum the threads are comparatively thick and coarse, but those growing in bouillon are very slender and delicate. The main trunk also is often thicker than the branches. When unstained they are homogeneous gray threads, without any appearance of a central canal or double-contoured wall. There is never any segmentation of the threads. When properly stained there is always a distinct beading or fragmentation of the protoplasm, but overstaining with fuchsin produces rather coarse, evenly red rods. The branching is irregular and without symmetry, and the branches are placed at a wide angle, very nearly, and sometimes quite, at right angles. This is best seen in specimens taken from liquid media. The irregularly stellate arrangement of the branches, which was observed by Eppinger in his original specimen, is often seen in young organisms floated out from a liquid medium.

Spore Formation.—On examining the deep-orange or red-colored growth upon potato, one is surprised to find that the threads have entirely disappeared and that the specimen consists of moderately large cocci. These cocci represent the spore form of the organism, and when planted upon blood serum the branching threads again appear. The spores stain readily with carbol-fuchsin and are not easily decolorized. They are spherical, or nearly so, but often appear somewhat elongated, apparently from beginning germination. They are killed by exposure to moist heat of 65° to 70° C. for an hour, but are more resistant to dry heat. Drying destroys the threads after a comparatively short time, but the spores retain their vitality for an indefinite period. A dried-up potato culture retains its vitality at the end of almost four years.

The identity of this microörganism is not fully established. It is undoubtedly a nocardia, but it does not agree in all particulars with any of the varieties described.

Animal Inoculations.—A number of rabbits and guinea-pigs were inoculated subcutaneously upon the abdomen and in the neighborhood of the cervical, axillary, and inguinal lymph nodes with colonies broken up in salt solution. Indurated swellings were produced at the point of inoculation and a number of abscesses resulted. The abscesses developed rapidly and some of them opened spontaneously,

while others were incised. The material evacuated did not resemble ordinary pus, but was thick and mucilaginous and exceedingly tenacious, like that from the subcutaneous abscesses of the patient described above. The microscopic appearance was the same, and the nocardia threads were found in considerable numbers. Several rabbits and guinea-pigs and two cats received peritoneal inoculations, but none of them showed any sign of infection. When rabbits were inoculated intravenously, a rapidly fatal general infection was produced, and the lesions were similar in kind and distribution to those described in the human subject.

Other Cases Reported.—*Ferré* and *Faguet* found in Bordeaux, in a cerebral abscess in the centrum ovale, a branching fungus, colored by Gram, which corresponded to nocardia. It grew on agar in round, ochre-colored colonies; on potato there was little growth visible; slimy, tough colonies, which became gray and remained free from white dusting on the surface. Inoculations in rabbits and guinea-pigs were negative.

Varieties of nocardia have been found in the human vagina. We have found a variety of nocardia in several cases of still-birth with infection of the placenta with the same organism. The organism is being studied.

Nocardia in Cases Simulating Actinomycosis or Tuberculosis.—*Sabraces* and *Rivière* found, in a case of cerebral abscess and a case of chronic lung disease with occurrence of subacute abscesses, fungi which differed from actinomyces. The organisms were contained in the lungs and pus in the latter in pure culture. They grew best at 37° C. in the presence of oxygen. On agar plates round, wart-like colonies were found with yellowish under and whitish upper surface. Grew particularly well on fat and glycerin media; in milk a flesh-colored rim was developed; in gelatin agar a rough, brownish deposit, becoming black with age. Gelatin was liquefied. The culture had a strong odor of old mould. A yellowish pigment was usually produced which dissolved in ether; in an atmosphere of pure oxygen a brown pigment. Animal experiments gave positive results only when to a fourteen-day-old bouillon culture lactic acid was added; then pseudotuberculosis was produced.

Numerous cases have since been observed in which nocardia proved to be the cause of chronic lung diseases, clinically suspected to be tuberculosis.

Treatment.—Recently homologous vaccines have been tried in certain cases, but it is yet too soon to determine with what result.

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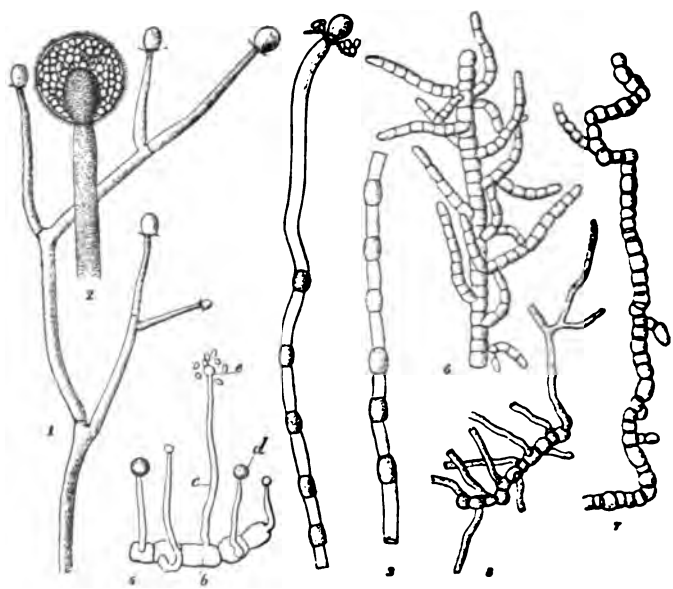
CHAPTER XXXVI.

THE PATHOGENIC MOULDS (HYPHOMYCETES, EUMYCETES) AND YEASTS (BLASTOMYCETES)—DISEASES DUE TO MICRO-ORGANISMS NOT YET IDENTIFIED.

THE HYPHOMYCETES.

THE majority of the moulds are not pathogenic and interest us merely as organisms which are apt to infect our bacteriologic media. Some are, however, true parasites, and produce a number of rather common diseases; for example, ringworm, favus, thrush, and pityriasis versicolor. Certain of the commoner moulds have also been reported from time to

FIG. 153



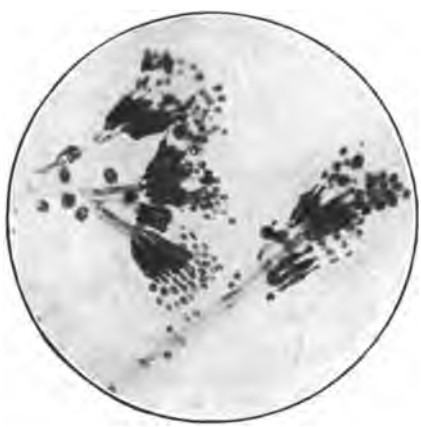
Chlamydomucor racemosus: 1, branched hypha carrying sporangia; 2, cross-section of sporangium highly magnified showing (*a*) sporangium carrier; (*b*) columella, and (*c*) spores; 3 and 4, chlamydospore building; 5, developing chlamydospores and sporangium (*e*); 6 and 7, branching; 8, sprouting spores. (After Brefeld.)

time as present in pathologic conditions in man as well as in the lower animals. Many varieties have been found in plant diseases, and others indirectly may be a source of danger to man. Indeed, when they form poisonous substances, as in the infection of grain by claviceps purpurea (ergot poisoning), they are distinctly dangerous.

The relation of the moulds to the bacteria is shown on p. 458. Like the higher bacteria, these organisms grow in filaments, but the majority

of them show more complicated structure in possessing a more distinct wall and a definite nucleus and in their reproductive organs. Each filament is termed a hypha. The hyphæ branch and grow into a dense network called mycelium. In the lower forms each hypha is a single cell, septa only occurring when fructification begins, while in the higher forms the filaments are composed of rows of cells. Most of the varieties form endospores in special spore sacks or sporangia, produced by the end swelling of a hypha (Fig. 153). In certain varieties a primitive sexual process has been observed, a conjugation of two cells with the formation of a zygospore, from which a sporangium

FIG. 154



Penicillium glaucum. Gelatin culture. Spread stained with gentian-violet. 500 : 1. (From Itzerott and Niemann).

carrier may arise and immediately develop a sporangium. Spores may also be produced in so-called gummæ (chlamydospores), which are swollen portions, segmented in the course of a hypha. (Fig. 153). Finally spores may be formed as conidia (Fig. 154).

The common moulds grow easily, especially in an acid medium, hence they are often found on fruit. The more pathogenic varieties grow with more difficulty. Among the common moulds, various species of mucor and of aspergillus have been reported pathogenic for man. Paltauf reported the case of a man who died after enteritis with secondary peritonitis. The autopsy showed multiple abscesses in brain and lungs, besides the lesions in the intestines and peritoneum, in all of which a species of mucor was found. Two other cases of primary mucor infection in humans were reported by Furbringer. A number of species of mucor have been found in ear and eye infections; for example, *Mucor corymbifer* (Fig. 155) has been found in ophthalmia. A number of varieties are pathogenic for lower animals.

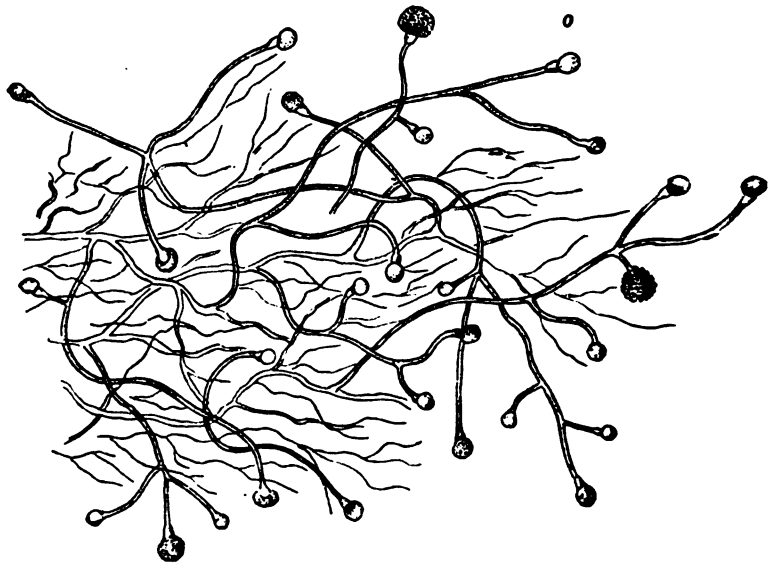
Aspergillus is found still more frequently in lower animals, especially in birds, where a kind of pseudotuberculosis is often produced. Quite a number of similar cases have been reported in man, and it

is supposed that the infection may be carried from birds to man. *Aspergillus fumigatus* (Fig. 156), is the most frequent variety found.

Penicillium minimum (similar to glaucum, Fig. 154) has been found by Liebermann in inflammation of the ear. The more common pathogenic forms for man are those producing the various hair and skin lesions mentioned above. These will now be described.

Trichophyton (Ringworm Fungus).—Ringworm of the body or hairless parts of the skin, *Tinea circinata*, and ringworm of the hairy parts, *Tinea tonsurans* and *Tinea barbæ* or *Tinea sycosis*, are due

FIG. 155



Mucor corymbifer, Cohn. Mycelium with underlying branched carriers. The sporangia at *o* have burst. $\frac{2}{1}$¹. (After Lichtheim.)

to the fungus *trichophyton*, discovered by Gruby in the human hair, and between the epidermal cells by Hebra, and obtained in free cultures by gravity.

According to Sabouraud, whose conclusions are based on an extensive series of microscopic examinations of cases of tinea in man and animals, of cultivation in artificial media, and of inoculation on man and animals, there are two distinct types of the fungus trichophyton causing ringworm in man—one with small spores (2 to 3 mm.) which he calls *T. microsporon*, and one with large spores (7 to 8 mm.) which he calls *T. megalosporon*. They differ in their mode of growth on artificial media and in their pathological effects on the human skin and its appendages. *T. microsporon* is the common fungus of Tinea tonsurans of children, especially of those cases which are rebellious to treatment, and its special seat of growth is in the substance of the hair. *T. megalosporon* (Fig. 157) is essentially the fungus of ring-

worm of the beard and of the smooth part of the skin; the prognosis as regards treatment is good. One-third of the cases of *T. tonsurans* of children are due to trichophyton megalosporon. The spores of *T. microsporon* are contained in a mycelium; but this is not visible, the

FIG. 156

Aspergillus fumigatus. Gelatin culture. Spread stained with gentian-violet. 500 : 1 (From Itserott and Niemann.)

spores appearing irregularly piled up like zoöglœa masses; and, growing outside, they form a dense sheath around the hair. The spores of *T. megalosporon* are always contained in distinct mycelium filaments, which may either be resistant when the hair is broken up or fragile and

FIG. 157

Hair riddled with ringworm fungus. Megalosporon variety.

easily breaking up into spores. The two types when grown in artificial cultures show distinct and constant characters. The cultures of *T. microsporon* show a downy surface and white color; those of *T. megalosporon* a powdery surface, with arborescent peripheral rays, and often a

yellowish color. Although the morphological appearances, mode of growth, and clinical effects of each type of trichophyton show certain characters in general, yet there are certain constant minor differences which point to the fact that there are several different kinds of species of fungus included under each type. The species included under *T. microsporon* are few in number, and, with the exception of one which causes the common contagious "herpes" of the horse, almost entirely human. The species of *T. megalosporon* are numerous and fall under several natural groups, the members of which resemble one another both from clinical and mycological aspects (Fig. 158). Many

FIG. 158

These two half-plates show three months' growth on peptone-maltose agar of two megalosporon varieties of the ringworm fungus. Natural size.

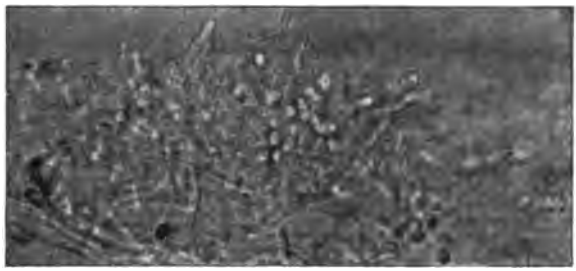
animals are subject to the growth upon their skins of particular varieties of *T. megalosporon*.

Achorion Schoenleinii (Favus).—Favus is due to a fungus discovered by Schoenlein in 1839, and called by Remak *Achorion schoenleinii*. The disease is communicated by contagion, the fungus being often derived from animals, especially cats, mice, rabbits, and fowls; dogs also are subject to it. It grows much more slowly than the ringworm fungus, and is, therefore, not so easily transmitted. Want of cleanliness is a predisposing factor. The fungus seems to find a

more favorable soil for its development on the skin of persons in weak health, especially from phthisis, than in others.

Pathologically, the disease represents the reaction of the tissues to the irritation caused by the growth of the fungus. The spores generally find their way into the hair follicles, where they grow in and about the hair (Fig. 159). The favus fungus grows in the epidermis, the density of the growth causing pressure on the parts below, thus crushing out the vitality of the hair and giving rise to atrophic scarring. The disease shows a marked preference for the scalp, but no part of the skin is exempt, and even the mucous membranes are liable to be attacked. Kaposi has reported a case in which a patient suffering from universal favus died, with symptoms of severe gastrointestinal irritation, which was found after death to be due to

FIG. 159



A portion of a favus-infected hair; magnified.

the presence of the favus fungus in the stomach and intestines. On the scalp it first appears as a tiny sulphur-yellow disk or *scutulum*, depressed in the centre like a cup and pierced by a hair. This is the characteristic lesion. The cup shape is attributed by Unna to growth at the sides proceeding more vigorously than at the centre.

The favus fungus is readily cultivated at the body temperature, and also at room temperature, in the ordinary culture media, as agar, blood serum, gelatin, bouillon, milk, infusion of malt, eggs, potato, etc. (Fig. 160). The growth develops slowly and shows a preference to growth beneath the surface of the medium—except on potato, upon which it develops on the surface in layers. The characteristic form of growth is that of moss-like projections from a central body. The color is at first grayish-white, then yellowish. As seen under the microscope, ray-like mycelium filaments are developed, which divide into branches. The ends are often swollen or club-shaped, and there are various enlargements along the body of the filament.

Pityriasis Versicolor.—This organism belongs to a group of fungi which, in contrast to the more parasitic fungi, favus and trichophyton, invades only the most superficial layers of the skin. It does not penetrate the deeper layers nor does it give rise to any considerable

pathological changes in the skin or hair. Although the vegetative elements of these fungi are much more numerous in the affected portions of the skin than is the case with the more parasitic species, they are not nearly as contagious as the latter.

By preference *Pityriasis versicolor* attacks the chest, abdomen, back and axillæ; less frequently neck and arms, while exceptionally it attacks also the face. The growth shows itself as scattered spots varying in color from that of cream-coffee to reddish-brown. The spots are readily scraped off and show fine lamellation or scaling. Occasionally the spots are confluent, and sometimes arranged in ring form like *Herpes tonsurans*.

In spite of their slight contagiousness this is one of the most frequent dermatomycoses. Although it is distributed widely over the earth, it is more frequently observed in southern than in northern countries.

FIG. 160

Five-months-old colony of favus on peptone-maltose agar; actual size.

Persons with a tender skin and a disposition to perspire freely are particularly affected by *Pityriasis versicolor*, and this is undoubtedly the only reason why the affection is so frequently observed in consumptives. Women are more frequently attacked than men, while children and old people are rarely affected.

The source of infection is unknown, since the absence of contagion has frequently been demonstrated. It seems likely that the spores of this fungus are so widely distributed that susceptible individuals are easily infected.

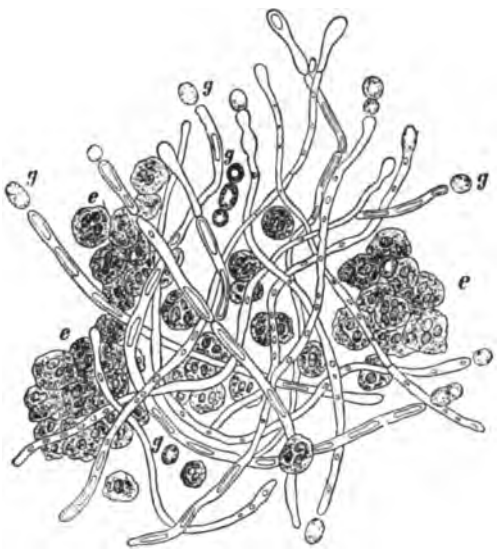
The arrangement of the fungus in the scales of epidermis is characteristic. The short and thick-curved hyphæ (7μ to 13μ long and 3μ to 4μ wide) surround large clumps of spores. The spores are coarse, doubly contorted (4μ to 7μ) or round. On staining with Ziehl's solution the spores are seen to contain deeply stained globules lying, in all probability, on the inner surface of the cell membrane. The rest of the protoplasm is but little stained, or not at all. One frequently finds that these globules have disintegrated into numerous fine granules. The globules are also found free; what their nature is does not appear; they are not found in cultures, the freshly developed spores showing only a single globular mass of protoplasm possessing a fine blue lustre.

Soor Fungus (**Thrush**) (Fig. 161).—Soor, as is well known, occurs most frequently in the oral mucous membrane of infants during the early weeks of life. It is also found as a slight mycosis in the vagina, especially of pregnant women. In rare cases the disease attacks adults, and then especially those whose system has been undermined by other diseases, such as diabetes, typhoid patients, etc. A few cases are recorded in the literature in which this fungus has given rise to constitutional disease. In these cases autopsy has shown

abscesses in various parts of the body, such as in the lungs, spleen, kidney, and brain.

In the lesions of the disease as well as in cultures, this fungus appears both as a yeast and a mycelium. It thus seems to stand between the true moulds and the yeasts. The yeast cells are oval in form, about 5μ to 6μ long and 4μ wide, and can in no way be distinguished from other yeast cells either by their appearance or their method of propagation.

FIG. 161



Inflammation of cornea by thrush. *g*. Conidia; *e*. pus cells. (From Plaut in Kolle and Wassermann.)

The threads of the mycelium vary markedly in length and thickness, and show all intermediate forms between a typical and a budding mycelium.

Soor is not much influenced by acids or alkalies, growing well both in acid and in alkaline media. On the other hand, it is very susceptible to the common disinfectants, especially salicylic acid, corrosive sublimate, phenol, etc. This fact is made use of in local treatment.

BLASTOMYCETES (YEASTS).

These microörganisms have been for many centuries of the greatest importance in brewing and baking (p. 102). They are not uncommonly present in the air and in cultures made from the throat. Certain recent experiments have shown that some varieties when injected are capable of producing tumor-like growths. Certain varieties are pathogenic for mice, and in recent years (since 1894) there have been reported a number of cases (twenty-three) of human infection from yeasts.

The position which the yeasts occupy in systematic biology has not, thus far, been accurately determined. In fact, it is even undetermined by some whether they constitute distinct fungi or whether they should be classed under the moulds.

The chief characteristic of the yeasts is their peculiar method of reproduction which in most cases is by means of budding. For this reason these organisms go by the name of *blastomycetes* in contrast to the fission fungi, or *schizomycetes*, and the thread fungi, or *hyphomycetes*. The fact was mentioned above that a transition between the blastomycetes and the hyphomycetes is formed by the *soor fungus*, which at one time grows to long threads, at another time (under certain conditions almost exclusively) multiplys by building. But no hard-and-fast line exists between these classes, for the yeasts can at times develop short hyphæ, at other times, in rare cases, form new individuals by segmentation.

The most important property of yeasts, though one not possessed by all to the same degree, is that of producing alcoholic fermentation. In practice we distinguish between the yeasts that can be employed practically, "culture yeasts," and those which often act as disturbing factors, so-called "wild" yeasts.

The shape of most of the culture yeasts is oval or elliptical (Fig. 162). Round or globular forms are more often met with among the wild species which usually excite only a slight degree of fermentation. They are known as "torula" forms. But sausage-shaped and thread forms are also met with.

The individual yeast cells are strongly refractive, so that under the microscope at times they have almost the lustre of fat droplets. This is important because in examining fresh tissues the yeast cells may be hard to distinguish from fat droplets, often requiring the aid of certain reagents for their identification.

The size of the individual yeast cells varies enormously, even in those of the same species or the same culture. In old colonies individuals may be found hardly larger than cocci, 1 to 2μ in diameter, while in other colonies, especially on the surface of a liquefied medium, giant yeast cells are found often attaining a diameter of 40μ or more. In spite of these wide fluctuations, however, the various species are characterized by a fairly definite average in size and form. Each cell contains a definite nucleus, which is demonstrated by the usual chromogenic stains.

During the process of budding the nucleus of the cell moves toward the margin, where it divides. At this point the limiting membrane of the cell ruptures or usually a hernia-like protrusion develops which has the appearance of a button attached to the cell. The daughter-cell so formed rapidly increases in size and gradually assumes the shape and size of the mother-cell.

A fact of the utmost importance for the propagation of the blastomycetes and continuation of the species is the formation of spores (Fig. 162). In this also the cell nucleus takes part, dividing into several frag-

ments, each of which becomes the centre of a new cell lying within the original cell. These new cells possess a firm membrane, a cell nucleus, and a little dense protoplasm. The number of spores developed in the yeast cells varies, but is constant for a given species. As a rule, one cell does not produce more than four endogenous spores, so-called astrospores; but species have been observed—*e. g.*, *Schizosaccharomyces octosporus* (Beijerinck)—in which eight spores are found.

Fig. 162

Saccharomyces cerevisiæ, I., Hansen. First stages of development of spores. $\frac{1}{1000}$. (From Hansen.)

Guilliermond has described conjugation in yeasts before the formation of spores.

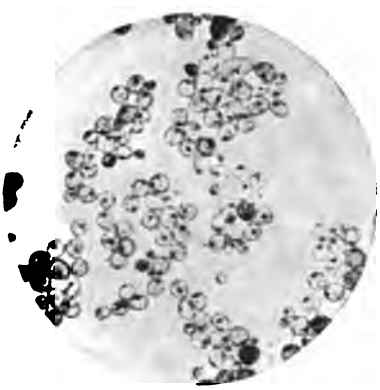
The vitality of yeasts is truly enormous. Hansen as well as Lindner were able to obtain a growth from cultures twelve years old. Busse succeeded in getting a luxuriant growth from a dry potato culture seven and a half years old, which was almost as hard as bone.

The pathogenic blastomycetes may be briefly summarized as follows:

Saccharomyces Busse, isolated in 1894 by O. Busse from the tibia of a thirty-one-year-old woman, who died thirteen months after the first symptoms appeared. The autopsy showed numerous broken-down nodules on several of the bones, in the lungs, spleen, and kidneys. The yeast was cultivated from all these foci (Fig. 163).

Saccharomyces tumefaciens, isolated in 1895 by Curtis. The patient was a young man showing multiple tumors on the hips and neck having the gross appearance of softened myxosarcomata.

Fig. 163



Saccharomyces Busse. × 350 diameters. (From Kolle and Wassermann.)

This yeast is pathogenic for rats, mice, and dogs, only slightly so for rabbits, and not at all for guinea-pigs.

Various similar cases have since been described, a number of them becoming generalized, and ending fatally. In generalized blastomycosis the lung seems frequently to be the seat of primary infection.

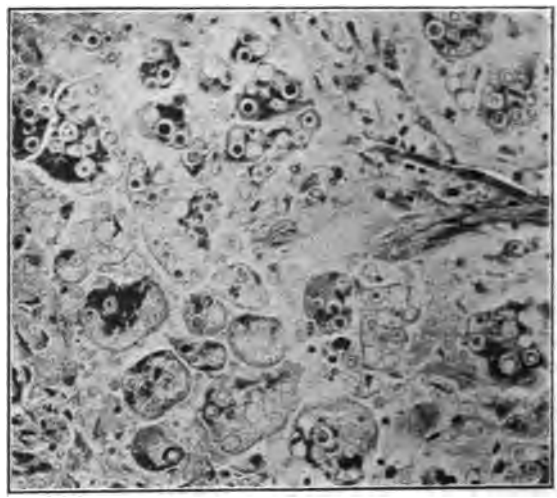
The cases described first by Rixford and Gilchrist as coccidiosis due to "*Coccidioides imitis*"—thought to be a protozoön—must be classed here, since Ophüls and others have shown that the "coccidium" formed hyphæ and elliptical forms on culture. Cultures from fresh tissue do not grow readily, but after they are once started they grow with ease on the usual laboratory media. The growth is more mould-like than yeast-like, except on potato when budding yeast-like cells are produced. Dogs,

rabbits, and guinea-pigs are susceptible to the fungus and show lesions similar to those in human beings.

A typical case of systemic blastomycosis has just been reported by Fontaine, Hasse, and Mitchell from Memphis, Tenn., accompanied by very good illustrations of tissue sections. Fig. 164 shows the characteristic microscopic appearance of the lung lesion.

Lundsgaard reported a case of ophthalmia due to a yeast. His patient, a man thirty-four years old, had a severe hypopyon keratitis, in the pus of which many yeast cells were present. Pure cul-

FIG. 164



Section of lung. ×150; blastomycetes in large syncytial cell masses. (From Fontaine, Hasse, and Mitchell.)

tures of these inoculated into guinea-pigs produced abscess both at the site of inoculation and in the lymph glands.

Buschke isolated a yeast from a cervical discharge in which no gonococci were present. The yeast was pathogenic for guinea-pigs.

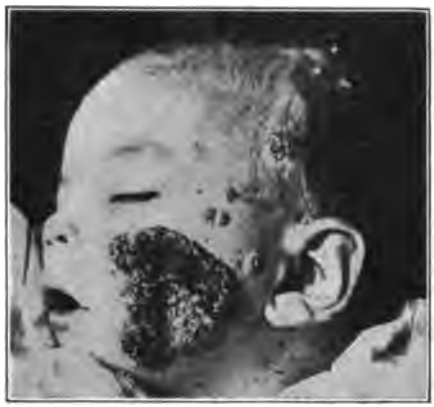
In 1895 Dr. G. Tokishige reported an epidemic quite common among horses in Japan, known as "Japanese worm," "benign worm," or "pseudoworm," which is caused by one of the saccharomyces. This disease begins in the skin in the form of hard, painless nodules from the size of a pea to that of a walnut. These break down and give rise to gradually extending ulcers. Pure cultures of the saccharomyces are pathogenic only for horses, not for rabbits, guinea-pigs, or hogs. In the districts where the disease prevails among horses it is also frequently seen in cattle.

Shortly after Tokishige's publication a similar disease occurring in horses in Italy and southern France was identified as being caused by saccharomyces. Cultures of this yeast, however, differ somewhat from that obtained in Japan, so that Busse is inclined to regard the two as two different species of blastomycetes.

Recently Kartulis, in Alexandria, has described about a hundred cases of a skin affection occurring in the gluteal regions of men and characterized by an elongated finger-like swelling, which breaks and emits a purulent discharge forming an unhealed sinus. In the discharge and surrounding tissues are numerous blastomycetes which Kartulis after cultivation and study considered a variety of the ordinary fermenting yeast (Saccharomyces cerevisiæ—Hansen). The cases were cured by excising the growth.

Kessler reported a skin lesion in an infant (see Fig. 165) probably due to a similar blastomycete, since the lesions healed after treatment with potassium iodide. The description of the yeast isolated is too incomplete to identify it.

FIG. 165



Blastomycosis in infant of 8 months, showing lesion on left cheek. (Kessler.)

Some years ago the attempt was made to connect the development of cancerous growth with blastomycetes. This was due in a measure to a certain similarity between the yeasts and the cell inclusions or so-called "parasites" of cancer, and, further, to the fact that when yeasts are injected into the animal body tumor-like nodules are often developed at the site of inoculation and in the internal organs. But these nodules are not tumors in the pathological sense of the term, but merely masses of blastomycetes mixed with inflammatory tissue proliferations to a very variable degree. At the present time Sanfelice and his pupils are perhaps the only ones who regard the thickenings produced in the tissues by *Saccharomyces neoformans* as true tumors. His work, however, is not at all convincing.

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DISEASES IN WHICH THE MICRO-ORGANISMS EXCITING THEM ARE AS YET UNDETECTED.

Measles.—Many bacteria as well as bodies supposed to be protozoa have been described by various investigators as occurring on the mucous membranes or in the blood of those sick of measles. None of these have been established as the exciting factor. For recent work, see p. 620.

Scarlet Fever.—Both streptococci and protozoa have been described as the exciting factors in this disease. The streptococci are certainly present, but are looked upon by most as secondary invaders. They undoubtedly add greatly to the gravity of the disease. The bodies described by Mallory as protozoa are still under investigation, and will be described in the section on Protozoa. Serum treatment has been used to overcome the streptococcus infection. The best results have been obtained in Vienna, and by Moser. He uses a serum obtained from horses receiving multiple cultures from cases of scarlet fever. Only about one horse in three gives a sufficiently curative serum. The doses used are very large (100 to 200 c.c.). The results claimed are very striking.

Typhus Fever.—Nothing has as yet been determined concerning the microörganisms exciting this disease. For work on the typhus fever of Mexico see p. 427.

Smallpox.—Streptococci as secondary invaders add here, as in scarlet fever, a dangerous infection. The status of protozoa is described fully under the section on Protozoa.

Rabies (Hydrophobia).—No bacteria have been discovered that are considered as factors. The probability of the Negri bodies being protozoa and the exciting factor is considered under Protozoa. The virus of rabies has been shown to be partially filterable (Remlinger, Bertarelli, and others) through the coarser Berkefeld filters. The retained portion is always more virulent than the filtrate. This indicates that there are some forms just within the limits of visibility and others larger, which corresponds with what we know of the variations in size of the Negri bodies (see p. 626).

Whooping-cough.—Jochmann and Krause (1901), in Germany, and Wollstein, in this country, have shown that bacilli differing slightly in cultural reactions and in agglutination from typical influenza bacilli can be detected in practically all cases of whooping-cough during the acute stages. Wollstein proved that the blood of cases of whooping-cough agglutinated these bacilli frequently in dilutions of 1 : 200 and over. Bordet and Gengou (1906) described a bacillus differing slightly from this, which they consider the specific organism because they obtain with it the complement fixation reaction. Wollstein (1909) was not able fully to corroborate their work.

Pemphigus Neonatorum.—Several micrococci have been described as the cause of infection.

Impetigo Contagiosa.—The findings have been similar to those in pemphigus.

Scurvy.—This disease is probably not due to microörganisms.

Mumps.—Diplococci have been considered by several investigators as possibly being the exciting organisms.

Noma.—It is as yet undecided whether this disease is due to one or to several microörganisms. A special predisposition of the tissues is necessary. A streptothrix, pseudodiphtheria bacilli, and diphtheria bacilli have been the organisms most frequently present.

Articular Rheumatism.—The specific organisms of this disease have been sought in the synovial fluid, blood, vegetations on heart valves, and in the exudates on tonsils, etc. Streptococci have been, of all bacteria, most frequently found. They grow in short chains or as diploccocci. Most bacteriologists believe the exciting factor has not yet been identified and that the streptococci and other cocci are important secondary infections.

Beriberi.—Microörganisms, both of bacterial and protozoan nature, have been considered as the exciting factor, but nothing definite has been proven.

Pellagra.—This disease has been much studied recently. The theory that it is due to the ingestion of damaged corn has received added evidence. Perhaps because of this fact or perhaps because it is true the disease seems to be on the increase. Whenever there is defective development of corn there is an increase in the prevalence and severity of the disease, while with the use of well-dried and healthy corn the disease decreases. By some observers the specific cause is supposed to be an intoxication similar to that of ergot poisoning, but others think it is a microörganism.

Invisible or Ultra-microscopical Organisms.—There exists a class of infectious diseases from which it has been quite impossible up to the present time to demonstrate visibly any microörganism, although infective material from such diseases may, with certain precautions, be passed through stone filters of varying degrees of porosity; the filtrates will contain the virus and be capable of reproducing the disease with all its characteristic symptoms when inoculated into a susceptible animal.

Examined microscopically, even with the highest powers, the filtrate is limpid, and, except in one or two diseases, which will be described in detail later on, not the faintest sign of particulate matter can be seen.

Certain precautions must be observed in such filtrations. In the first place, the filter must be shown by actual test to be free from imperfections—any and all of the known bacteria must be absolutely retained and none pass into the filtrate. (The bacillus of guinea-pig pneumonia, which is $0.5\mu \times 0.7\mu$, passes Berkefeld No. 5 (Wherry); a spirillum isolated by von Esmarch passes, according to him, Berkefeld, Chamberland F, and other filters; finally, a minute water flagellate was found by Borrel to pass through the coarser filters.) The filtration must be completed within a moderate time, because even bacteria as large as the typhoid bacillus may, in media containing a certain amount

of albuminous material, grow through the filter. The material to be filtered should be greatly diluted and first filtered through filter-paper in order to avoid the clogging action of extraneous material.

If, after all the proper precautions have been taken, the filtrate is pathogenic, one must be certain that the symptoms are due to a microörganism and not to a toxin—this may be decided with almost absolute certainty by inoculating a series of animals successively with the filtrate obtained from a previously so inoculated animal—it is impossible with our present knowledge to conceive that toxin or enzyme can be potent enough to cause symptoms after the enormous dilution it receives in passage through the animals, using a filtered virus for the first animal and a filtered virus for the second obtained from a portion of the infected material of the first.

Among the best known ultra-microscopical diseases are:

Anterior Poliomyelitis.—Recent Experimental Study.—Landsteiner and Popper[1] reported the transmission of acute poliomyelitis to apes. They inoculated spinal cord intraperitoneally and produced typical symptoms and lesions, but did not succeed in transmitting from ape to ape, probably because they used a mild case. They thought the virus belonged to the class of invisible protozoa.

Knoepfelmacher also failed to retransmit the disease, probably because he used a chronic case.

Flexner[2] transmitted the disease from monkey to monkey by means of intracerebral inoculations.

Landsteiner and Levaditi[3] also transmitted it from monkey to monkey. They found that virus lives four days outside of body; that the degenerative nerve cells are taken up by the phagocytes, and that there is an analogy between the lesions of poliomyelitis and those produced by rabies from street virus, as well as that it is filterable.

Leiner and Weisner transmitted the disease from monkey to monkey, found young animals more susceptible than older ones, and the spinal fluid, blood, and spleen negative.

Flexner[4] transmitted the disease by means of inoculation into the blood or peritoneal cavity, and transmitted the disease by means of the subcutaneous method, and found the virus to be filterable. Cultures so far have been negative.

Landsteiner and Levaditi[5] found the virus in the salivary glands and suggested the saliva, moist or dry, as a source of contagion.

Foot and Mouth Disease.—A highly infectious disease of cows. Other domestic animals, as well as man, may also be attacked, the latter becoming infected by drinking the milk of animals suffering

[1] *Landsteiner and Popper.* May 25, 1909, Zeitschrift für Immunitätsforschung, Band ii, H. 4.

[2] *Flexner.* Journal Amer. Med. Assn., Nov. 13, 1909.

[3] *Landsteiner and Levaditi.* Nov. 27. Compt. Rendus Soc. de Biologie, Dec. 3, 1909.

[4] *Flexner.* Journ. Amer. Med. Assn., Dec. 4 and Dec. 18.

[5] *Landsteiner and Levaditi.* Compt. Rendus Soc. de Biologie, Dec. 24, 1909, (read Dec. 18, 1909).

from this disease. It is characterized by the appearance of vesicles in the mouth, around the coronet of the foot as well as between the toes. The organism was discovered by Löffler and Frosch in 1898, who obtained it as follows: after diluting the contents of an unbroken vesicle with 20 to 40 times its volume of water, the resulting fluid was passed through a Berkefeld filter. The filtrate contains the virus and remains infectious for some time.

Yellow Fever.—The undiluted serum from cases of this disease has been shown by the American commission and others (see p. 638) to pass Berkefeld and Chamberland F filters and in this form to be infectious; therefore some forms at least of the specific organism are probably ultramicroscopic.

Mosaic Diseases of Tobacco.—The young leaves become devoid of chlorophyll in spots, which enlarge, turn brown, and the underlying tissue becomes necrotic. Beijerinck in 1899 showed that the filtrate from a porcelain filter promptly reproduced the disease on tobacco leaves, and he was inclined to believe the virus was in true solution.

South African Horse Sickness.—It occurs in warm weather, as a rule, and is said to be more common in animals which do not pass the night under cover. The horses are uneasy, have difficulty in breathing, and a reddish froth exudes from their mouth. The temperature rises in the daytime, but has a tendency to drop at night. In severe cases an œdematous swelling of the head and neck may appear. MacFadycan succeeded in passing blood serum of a diseased horse (diluted) through a Berkefeld and Chamberland F, but not through a B filter.

Rinderpest.—Rinderpest, the fatal European and African disease of cattle, is characterized by inflammation of the intestinal mucous membrane. The blood is infectious, and filtrates of it through Berkefeld and Chamberland F (Nicolle and Adel Bey) produce the disease. No organism can be seen.

Dengue.—Recently Ashburn and Craig claim to have reproduced dengue in susceptible individuals by a similar procedure to that employed in yellow fever. The virus passes a Berkefeld filter. The intermediary host in natural infection is claimed by them to be *Culex fatigans*.

In a certain number of diseases, at the highest limit of our present magnification, the cause of the disease is seen as minute granules. We know, surely in one disease at least, that these are the cause, because they have been made to grow and to produce the disease in new animals. This disease is known as

Contagious Pleuro-pneumonia of Cattle.—This malady affects bovines, but not other species. Typically there is an inflammation of the lungs and the pleura which is invasive and causes necrosis of the diseased parts. Nocard and Roux succeeded in cultivating the organism in collodion sacs placed in the peritoneal cavity of rabbits, using a mixture of serum and bouillon. After two weeks a very

faint turbidity appeared in the sacs: coincidently the fluid became infective. The organisms will pass a Berkefeld and Chamberland F filter, but not a Chamberland B.

Chlamydozoa (**Strongyloplasmæ**).—Lipschütz in a recent article calls attention to the fact, that though it can be seen, this organism will pass certain filters, and he claims to have discovered with Borrel similar organisms in moluscum contagio sum of man and birds. He calls them microscopically visible filterable organisms, and says that probably the organisms of vaccinia (Volpino's motile granules discovered in vaccinia by the ultramicroscope) and of rabies (Prowazek's chlamydozoa) belong to this class. He thinks they should be given the name "strongyloplasmen" because of their prevailing round form.

CHAPTER XXXVII.

THE BACTERIOLOGICAL EXAMINATION OF WATER, AIR, AND SOIL—THE CONTAMINATION AND PURIFICATION OF WATER—THE DISPOSAL OF SEWAGE.

THE bacteriological examination of water is undertaken for the purpose of discovering whether any pathogenic bacteria are liable to be present. The determination of the number of bacteria in water was for a time considered of great importance, then it fell into disrepute, and the attempt was made to isolate the specific germs of diseases which were thought to be water-borne. At first these attempts seemed very successful in that supposed typhoid bacilli and cholera spirilla were found. Further study revealed the fact that there were common water and intestinal bacteria which were so closely allied to the above forms that the tests applied did not separate them. Even the use of a serum from an animal immunized to injections of the typhoid bacillus was found to agglutinate some other bacteria in high dilutions; so that the test as usually carried out was insufficient. With the latest technique it is probable but not certain that absorption tests with the serum from an immunized animal will be sufficient to decide whether a suspected bacillus is the typhoid bacillus or not. The improbability of getting typhoid bacilli from suspected water except under unusually favorable conditions caused a return to the estimation of the number of bacteria in water and above all to the estimation of the number of intestinal bacteria. It is known that the group of colon bacilli have a somewhat longer existence than the typhoid bacilli, and as the colon bacilli come chiefly or wholly from the intestinal passages of men and animals, it was fair to assume that typhoid bacilli could not occur without the presence of the colon bacillus except in rare cases, as, for example, pollution with urine alone. The latter could, of course, occur abundantly without the typhoid bacillus.

During the past few years the attention of sanitarians has been seriously devoted to the interpretation of the presence of smaller or larger numbers of colon bacilli in water, until at present upon the quantitative analysis (measuring, within certain limits, decomposing organic matter) and the colon test (indicating more specifically that pollution derived from intestinal discharges of man or animals) the bacteriological analysis of water is based. The determination of the number of bacteria is also of value.

Technique for Quantitative Analysis.—The utmost care is necessary to get reliable results. A speck of dust, a contaminated dish, a delay of a few hours, an improperly sterilized agar or gelatin, a too high or too low temperature, may introduce an error or variation in results which would make a reliable test impossible.

Collection of Samples.—The small sample taken must represent the whole from which it was drawn. If a brook-water, it must be taken some distance from the bank; if from a tap, the water in the pipes must first be run off, for otherwise the effect of metallic substances will invalidate the results; if from lake or pond, the surface scum or bottom mud must be avoided, but may be examined separately. The utensils by which the water is taken should be of a good quality of glass, clean and sterile. From a brook the water can be taken directly into a bottle, the stopper being removed while it fills, avoiding the surface film and its attending excessive numbers of bacteria; from a river or pond it can be taken from the bow of a small boat, or from a bottle properly fastened on the end of a pole so as to avoid contamination; from a well a special apparatus has been devised by Abbott, where a bottle with a leaded bottom is so held that when lowered to the proper depth a jerk will remove the cork and allow the bottle to fill. The same device or another accomplishing the same purpose can be rigged up readily by anyone. The sample of water should be tested as soon as possible, for the bacteria immediately begin to increase or decrease. In small bottles removed from the light predatory microörganisms and many bacteria begin to increase, and among these are the members of the colon group. Thus, the Franklands record a case in which in a sample of well-water kept during three days at a moderate temperature the bacteria increased from 7 to 495,000; while Jordan found that in a sample the bacteria in forty-eight hours fell from 535,000 to 54,500. In a sample we took from the Croton River the colon bacilli during twenty-four hours increased from 10 to 100 per c.c. The only safe way to prevent this increase is to plate and plant the water in fermentation tubes within a space of one or two hours. It is far better to make the cultures in the open field or in a house rather than to wait six to twelve hours for the conveniences and advantages of the laboratory. If sent to the laboratory, water should be kept in transit at about 5° C. (41° F.).

The third matter of great importance is the adding of proper amounts of water to the broth in the fermentation tubes and the media for planting. Usually 1 c.c., 0.1 c.c., and 0.01 c.c. are added to the fermentation tubes and to 10 c.c. of the melted nutrient agar or gelatin. If possible duplicate tests should always be made. When it is desired to know whether colon bacilli are present in larger amounts than 1 c.c., quantities as great at 10 or 100 c.c. can be added to bouillon, and then after a few hours 1 c.c. added to fermentation tubes. Less than twenty colonies and more than two hundred on a plate give inaccurate counts, the smaller number being too few to judge an average and the larger number interfering with each other. When as many as 10,000 colonies develop in the agar contained in one plate, it will be found that there will develop in a second plate containing but one-tenth the amount of water from 20 to 50 per cent. as many colonies. This shows that the crowding of the colonies had prevented the growth of all but one-fifth to one-half of them.

The chemical composition of the medium on which the bacteria are grown affects the result of the analysis. Nutrient 1.5 per cent. agar gives slightly lower counts than gelatin, but on account of its convenience in summer and its greater uniformity it is being more and more generally used for routine quantitative work. There is an optimum reaction for every variety of bacteria, and to ensure uniformity the committee of the American Public Health Association adopted a standard reaction of +1.0 per cent., which was as near as possible to the average optimum for water bacteria. Such a uniform standard is a necessity to secure comparability of results. At best only a certain proportion of bacteria develop, and it is only important that our counts represent a section through the true bacterial flora which fairly represents the quick-growing sewage forms. Comparability is the vitally essential factor.

The temperature at which the bacteria develop is of great importance, and they should be protected from light. The access of oxygen which prevents the growth of anaërobes must also not be forgotten. As a rule, the plate cultures are developed for four days at 20° to 21° C., and for twenty-four or forty-eight hours at incubator temperature. Some bacteria do not develop colonies in four days, but these are neglected. The number of bacteria growing at room temperature is usually much greater than those growing at 37°. As all the intestinal groups of bacteria grow at body temperature, while many of the water types do not, some investigators believe it important to develop the bacteria at both temperatures so as to compare the results. We have not found this to be of any advantage when tests are also made for the colon group of bacilli.

The lactose broth is placed at 37° C. for the development of the colon bacilli. The fermentation tubes not showing gas are recorded as negative and usually discarded. Those showing gas are suspected to contain colon bacilli. To a number of tubes containing melted litmus-lactose agar at about 44° C. are added 1, 0.1, and 0.01 loop of the culture fluid. Plates are poured and the whole placed in the incubator. The *Bacillus coli* ferments lactose and thus produces acid, so that if colon bacilli are present we have a number of red colonies on a blue field. Later, if many colon bacilli were present, the whole medium becomes acid. At forty-eight hours, on account of alkali being produced by the formation of NH_3, the blue may return. If after inspection red colonies are seen, four or five are picked and planted into lactose bouillon and other media. Litmus-lactose agar is frequently used for the original plating of water samples, the absence or presence of acid-producing colonies being thus immediately noted. The colon-like cultures should be subjected to the Vosges reaction (page 499), and should be kept for 1 month at 20° C. in gelatin before a decision is made. Colon bacilli do not liquefy gelatin nor give the Vosges reaction. There are a few colon-like bacilli in the intestinal tract that give the Vosges reaction. For a more complete understanding of the technique and the interpretation

of results of the bacteriological examination of water see Elements of Water Bacteriology—Prescott and Winslow.

For the characteristics of the colon bacilli the Massachusetts State Board of Health uses six media—gelatin, lactose agar, dextrose broth, milk, nitrate solution, and peptone solution, determining, respectively, absence of liquefaction, production of gas, turbidity, coagulation without liquefaction of the coagulaum, products of nitrite, and indol.

Lactose-bile-peptone solution has been much used. In badly contaminated waters this has a distinct advantage in that the bile inhibits many varieties of bacteria more than those of the colon-typhoid group. In good waters the results are very similar from the lactose-peptone and lactose-bile-peptone solutions.

Significance of the Colon Bacillus.—The colon test has been received by the majority of engineers and practical sanitarians with great satisfaction, and has been applied with confidence to the examination not only of water, but of shell-fish and other articles of food as well. On the other hand, some have denied its value. Bacteriologists have found bacilli like certain members of the colon group in apparently unpolluted well-water. The discovery that animals have colon bacilli identical in the usual characteristics studied with those of man has complicated matters. Thus a fresh hillside stream may be loaded with colon bacilli from the washings of horse or cow manure put on the fields through which it runs or polluted by a stray cow or horse. Swine, hens, birds, etc., may contaminate in unsuspected ways. The number of colon bacilli rather than their presence in any body of surface water is therefore of importance. In well- and spring-water the presence of colon bacilli indicates contamination. The absence of colon bacilli in water proves its harmlessness so far as bacteriology can prove it. When the colon bacillus is present so as to be isolated from 1 c.c. of water in a series of tests, it is reasonable proof of animal or human pollution and the conditions should be investigated. Ten colon bacilli in 1 c.c. indicates serious pollution. Surface waters from inhabited regions will always contain numerous colon bacilli after a heavy rain storm or shower. The washings from roads and cultivated fields contain necessarily large numbers. Winslow reports that in only two out of fifty-eight samples of presumably non-polluted waters did he get colon bacilli in the 1 c.c. samples. Even in twenty-one stagnant pools he only found colon bacilli in five of the 1 c.c. samples.

The experience of all who have studied the subject practically is that in delicacy the colon test surpasses chemical analysis; in constancy and definiteness it also excels the quantitative bacterial count. All these tests must, however, be supplemented by inspection.

Interpretation of the Quantitative Analysis.—The older experimenters attempted to establish arbitrary standards by which the sanitary quality of water could be fixed automatically by the number of germs alone. This has been largely given up. Dr. Sternberg considers that a water containing less than 100 bacteria is presumably

from a deep source and uncontaminated by surface drainage; that one with 500 bacteria is open to suspicion; and that one with over 1000 bacteria is presumably contaminated by sewage or surface drainage. Even this conservative opinion must be applied with caution. The source of the sample is of vital importance in the interpretation; thus, a bacterial count which would condemn a spring or well might be normal for a river. In woodland springs and lakes several hundred bacteria per c.c. are frequently found. In lakes the point at which the sample is taken is of great importance, as the bacterial count varies with the distance from the shore and with the depth. The weather also is an influence, since the wind causes currents and waves which stirs up the bottom mud, bringing up organisms which have been sedimented. Rains greatly influence streams by flooding them with surface water bringing a huge number of bacteria at times. The season of the year is an important factor. The counts are highest in the winter and spring months, and lower from April to September.

The following figures illustrate this point:

Water	Observer	Year	Jan.	Feb.	March	April	May	June
New York City tap-water	Houghton	1904	890	1100	650	240	350	370
Boston tap-water	Whipple	1892	135	211	102	52	53	86
Merrimac River tap-water	Clark	1899	4900	5900	6300	2900	1900	3500

The winter and spring increases are not exceptions to the rule that high numbers indicate danger, but an indication of its truth; for it means a melting of the snow and a flow of surface water into the streams without the usual filtering soil filtration. A number of severe epidemics of typhoid fever have been produced in this way. It is only the fact that typhoid fever is at a minimum in winter that prevents more frequent pollution. Although, as a rule, a series of tests are necessary to pass judgment on a water, a single test may be very important. A large increase in the number in tap-water a day after a storm points unerringly to surface pollution, and if towns exist in the water-shed, to street and sewer pollution. The Croton water frequently jumps from hundreds to thousands after such a storm.

In a typhoid epidemic at Newport, Winslow reports that a test of the water supply showed but 334 bacteria per cubic centimetre, but one from a well thowed 6100. The suspicion aroused was justified by finding all the typhoid cases had gotten water from this well.

The study of the bacterial effluent from municipal water filters is the only way in which the efficiency of the filter and the accidents which occur can be determined. In Germany these regular tests are obligatory. The filter should remove about 99 per cent. of the bacteria. Elaborate studies have recently been made of the exact distribution of streams of sewage in bodies of water into which they flow, their disappearance by dilution and sedimentation, and their removal by death. Under peculiar conditions bacteria in water may increase for a time, but here the prevailing bacteria belong almost exclusively to one type.

Streptococci in Sewage.—The varieties of streptococci found most often in polluted water correspond to the streptococci described by Houston. In some water in which these are found no *B. coli* have been found and there is considerable doubt in such cases as to whether the streptococci imply serious pollution. The streptococci remain alive longer than the colon bacilli. In England the examination for streptococci in water is much more regularly done than in America.

Other Bacteria.—Most of the bacteria which develop in the intestines of man and animals necessarily occur in polluted water, and an examination for some of these has been advocated by many, such as the B. enteritidis sporogenes, other anaërobic spore formers, the various members of the typhoid-colon group, and the proteus group.

Isolation of the Typhoid Bacillus from Water.—If it were possible to readily obtain the typhoid bacilli from water, when they were present in small numbers, its examination for that purpose would be of much greater value than it is now; but we have to remember that we can only examine at one time a few cubic centimetres of water by bacteriological methods, and that although the typhoid bacilli may be sufficiently abundant in the water to give, in the quantity that we ordinarily drink, a few bacilli, yet it must be a very lucky chance if they happen to be in the small amount which we examine. Still, further, although it is very easy to isolate typhoid bacilli from water when they are in considerable numbers, yet when they are a very minute proportion of all the bacteria present it is almost impossible not to overlook them. Many attempts have been made to devise some method by which the relative number of the typhoid and other parasitic bacteria present in water could be increased at the expense of the saprophytic bacteria. Thus, to 100 c.c. of water 25 c.c. of a 4 per cent. peptone nutrient bouillon is added, and the whole put in the incubator at 37° C. for twenty-four hours. From this, plate cultures are made. As a matter of fact, the typhoid bacillus is rarely found, even in specimens of water where we actually know that it is or has been present because of cases of typhoid fever which have developed from drinking the water. From these facts we must consider our lack of finding the bacillus in any given cases as absolutely no reason for considering the water to be free from danger. Another serious drawback to the value of the examinations for the typhoid bacillus is that they are frequently made at a time when the water is really free from contamination, though both earlier and later the bacillus was present. It is hardly worth while, therefore, except in careful experimental researches, to examine the water for the typhoid bacillus, but rather study the location of the surrounding privies and sources of contamination. A number of observers, resting on the agglutination test, have thought they have isolated typhoid bacilli from the soil and water, but these investigators had not considered sufficiently the matter of group agglutinins, and their results are not trustworthy.

CONTAMINATION AND PURIFICATION OF DRINKING WATERS.

Brook-water and river-water are contaminated in two ways: through chemicals, the waste products of manufacturing establishments, and through harmful bacteria by the contents of drains, sewers, etc., the latter method being by far the more dangerous.

When water, which has been soiled by waste products of manufactories only, becomes so diluted or purified that the contamination is not noticeable to the senses and shows no dangerous products on chemical analysis, it is probably safe to drink. When sewage is the contamination, this rule no longer holds, and there may be no chemical impurities and no pathogenic bacteria found, and yet disease be produced. That river-water which has been fouled by sewage will, by oxidization, dilution, sedimentation, action of sunlight, and predatory microörganisms, become greatly purified is an indisputable fact. The increase in bacteria which occurs from contamination is also largely or entirely lost after ten to twenty miles of river flow. Nevertheless, the history of many epidemics seems to show that a badly contaminated river is never an absolutely safe water to drink, although with the lapse of each day it becomes less and less dangerous, nor will sand filter-beds absolutely remove all danger. These statements are founded upon the results of numerous investigations; thus the marked disappearance of bacteria is illustrated by the following: Kummel found below the town of Rosbock 48,000 bacteria to the cubic centimetre; twenty-five kilometres farther down the stream only 200 were present—about the same number as before the sewage of Rosbock entered. On the other hand, the doubtful security of depending on a river purification is proved by such experiences as the following: In the city of Lowell, Massachusetts, an alarming epidemic followed the pollution of the Merrimac River three miles above by typhoid fæces, and six weeks later an alarming epidemic attacked Lawrence, nine miles below Lowell. It is estimated that the water took ten days to pass from Lowell to Lawrence and through the reservoirs. Typhoid bacilli usually die in river-water in from three to ten days, but they may live for twenty-five days in other water; the Lawrence epidemic is easily explained. Newark-on-Trent, England, averaged seventy-five cases a year from moderately well filtered water and only ten when it was changed to deep-well supply.

Purification of Water on a Large Scale.—For detailed information on this subject the reader is referred to works on hygiene. Surface waters, if collected and held in sufficiently large lakes or reservoirs usually become so clarified by sedimentation, except shortly after heavy rains, as to require no further treatment so far as its appearance goes. The collection of water in large reservoirs allows not only the living and dead matter to subside, but allows time also for the pathogenic germs to perish through light and antagonistic bacteria and other deleterious influences, sand or mechanical coagulant. Filtration of water exerts a very marked purification, taking

out 99.8 per cent. of the organisms in those best constructed and at least 95 per cent. in those commonly used in cities. The construction of filters is too large a subject to enter on minutely here; sand filters consist, as a rule, of several layers, beginning with fine sand, and then smaller and larger gravel, and finally rough stones. A certain time elapses before the best results are obtained; this seems to wait for the formation of a film of organic material on the sand, which is full of nitrifying bacteria. Even the best filters only greatly diminish the dangers of polluted water. Spring-water and well-water are, in fact, filtered waters.

Water which is subject to serious pollution must be submitted to a preliminary purification before it can be considered a suitable source for a drinking-water supply. The means employed for its purification depend to a large extent upon the character of the water and the nature of the pollution. Filtration through slow sand filters, three to five feet in depth, removes 98 to 99.5 per cent. of the bacteria and organic matter; so that effluents from the best constructed sand filtration beds constitute safe and reliable drinking waters. Five hundred thousand to one or two million gallons, depending somewhat upon the extent of pollution and the fineness of the sand, can be filtered daily per acre. Only the surface of the sand filter becomes in any way clogged and as thin a layer as can be scraped off is removed one or more times a month. This surface sand is washed with clean water and several scrapings replaced at one time. Sand filtration beds are very widely used abroad and are coming into extensive use in this country. The filter-beds at Lawrence, Mass., have been used over ten years with marked success; when properly managed, they render the highly polluted Merrimac River a fairly safe drinking water; the filter-beds there are scraped about thirteen times a year.

Mechanical filtration plants find considerable favor where clarification as well as bacterial purification is desired. A coagulant such as sulphate of aluminum is employed and forms in the water a flocculent precipitate which carries down with it all suspended matter; 125,000,000 or more gallons of water can be filtered on an acre daily, but the filters must be washed daily by reversing the flow and cleansing the clogged filter with a stream of the purified water. Chlorinated lime when added to drinking water to the extent of one-eighth to one-twelfth of a grain per gallon will destroy all intestinal bacteria of the typhoid-colon group within a few hours. This is a very useful means of purification for emergencies. It does not injure the water.

Under special conditions other methods, such as the passage of ozone, have proved successful.

Domestic Purification.—Water which requires private filtering should not be supplied for drinking purposes. Unhappily, however, it often is. Domestic filters may be divided, roughly, into those for high and low pressure. The former are directly connected with the water main, while the others simply have the slight pressure of the

column of water standing in the filter. Many household filters contain animal charcoal, silicated carbon, etc., either in a pressed condition or in one porous mass. These filters remove much of the deleterious matter from the suspected waters, but the majority cannot be depended upon to remove all bacteria. Even those which are equipped for self-cleansing become in a little while foul, and, if not cleaned, unfit for use. The best of the filters are of porous stone, such as the Berkefeld and Pasteur filters. These yield a water, if too great a pressure is not used, almost absolutely free from bacteria, and if they are frequently cleansed they are reliable. A large Berkefeld filter will allow sixty gallons of water to pass per hour. The Pasteur filter is more compact and slower. From the best Pasteur filters sterile water may be passed for two to three weeks; from the Berkefeld usually only a few days. A single typical low-pressure filter is that of Bailey Denton. The upper compartment contains the filtering material, which may be sand or charcoal, and is fed from a cistern or hydrant. After a certain quantity of water has passed in, the supply is automatically cut off until the whole amount is filtered. A fairly efficient filter is the following: Take a large-sized earthenware pot and plug the hole in the bottom with a cork, through which pass a short glass tube. Upon the bottom place an inch of small pieces of broken flower-pot; upon this a couple of inches of well-washed small gravel, and upon this six to twelve inches of well-washed, fine, sharp sand. Cover the sand with a piece of filter-paper and hold this down with a few small stones. Mount the pot on a tripod, and it is ready for use. The paper prevents the sand being disturbed when water is added, and as it also holds most of the sediment, this can be readily removed. Every few months the sand can be washed and replaced. Animal charcoal is not a good substance for permanent filters, as bacteria grow well in it. Whenever water is suspected, and there is any doubt as to the filters, it should be boiled for ten minutes; this will destroy all bacteria. This precaution should always be taken in the presence of typhoid fever and cholera epidemics.

THE DISPOSAL OF SEWAGE.

The disposal of sewage is becoming a vital question with all towns and cities which are not situated near salt-water outlets, since the present tendency in legislation is to compel such towns to dispose of their waste so that it shall not be a menace to drinking-water streams, destructive to fisheries, or a nuisance to harbors.

Methods of sewage purification depend upon the character of the sewage and the kind of effluent desired.

Two hundred thousand gallons of crude sewage may be filtered upon an acre of land daily and an effluent obtained which will compare favorably in every way known to the chemist and bacteriologist with the best mountain springs. This is, however, a slow process, and it is rare that such a pure effluent is required. Similar results

may be obtained by utilizing the septic-tank method, running the sewage from the septic tank to contact beds and thence to sand filter-beds; where because of the partial "self-purification of the sewage" in the septic tank and contact beds 2,500,000 gallons of sewage can be filtered daily on an acre of surface. In this process less land is required and both these effluents can be safely turned into drinking-water streams.

If, however, a merely non-putrescible effluent is required, one which, though high bacterially, will not be offensive in any way, or subject to further decomposition, it may be obtained by passing crude sewage to septic tanks, thence to double contact beds, the resulting effluent having merely an earthy, humus-like odor and being non-putrescible.

Where acid wastes, tannery wastes, dyestuffs, etc., from various factories enter into sewage, its disposal becomes a more complicated problem and chemical precipitation by the use of lime or other chemicals is generally employed for such sewage purification, which at best is only partial and is sometimes supplemented by sand filtration.

Sea-water.—This is only feebly bactericidal. The salty tidal waters of rivers allow typhoid bacilli and other members of the typhoid-colon group to live for a number of days.

BACTERIOLOGICAL EXAMINATION OF AIR.

Saprophytic bacteria are always present in considerable numbers in the air except far out at sea or on high mountains. They are more abundant where organic matter abounds and in dry and windy weather. Pathogenic bacteria, on the other hand, are only occasionally present in the air. The practical results obtained from the examination of air for pathogenic bacteria have been slight. We know that at times they must be in the air, but unless we purposely increase their numbers they are so few in the comparatively small amount of air which it is practicable to examine that we rarely find them. Examination of dust, however, in hospital wards and sick-rooms, in places where only air infection was possible, have revealed tubercle bacilli and other pathogenic bacteria.

The simplest method of searching for the varieties of bacteria in the air and their number in any place is to expose to the air for longer or shorter periods nutrient agar spread upon the surface of the Petri dish. After exposure the plates are either put in the incubator at 37° C. or kept at room temperature. The more careful quantitative examination is made by drawing a given quantity of air through tubes containing sterile sand, which is kept in by pieces of metal gauze. When the operation is completed the sand is poured into a tube containing melted nutrient gelatin or nutrient agar, and after thoroughly shaking, the mixture is poured into a Petri dish and the bacteria allowed to develop, either at 37° or 23° C., according as the growth of the parasitic or saprophytic varieties is desired. Instead of agar or gelatin,

ascitic broth or animals may be inoculated. Such examinations are occasionally made of the air of theatres, crowded streets in cities, etc. They give interesting, but hardly valuable results.

BACTERIOLOGICAL EXAMINATION OF THE SOIL.

The subject from its agricultural side is considered on p. 95. Specimens of deep soil can be gathered in sterile, sharp-pointed, sheet-iron tubes. Through the examination, we wish to learn either the number of bacteria or the important varieties of bacteria present. To estimate the number, small fractions of a gram are taken and planted in nutrient agar or in special media contained in Petri dishes. Anaërobic as well as aërobic cultures should be made.

According to Houston, uncultivated sand soil averages 100,000 bacteria per gram, garden soil 1,500,000, and sewage-polluted 115,000,000. The most important bacteria to be sought for are bacilli of the colon group and streptococci. Both of these suggest fairly recent excremental pollution.

The period during which typhoid bacilli remain alive in soil is variable, since it depends on so many unknown factors and differs so in different places. The typhoid bacilli probably rarely increase in the soil and probably rarely survive a month in it. The main danger of soil bacteria is their being washed into water supplies by rains or carried to them by the wind.

Reaction of Vosges and Proskauer.—Grow the culture in 1 per cent. glucose-peptone water in a fermentation tube for four days at 37° C. Add 1 c.c. of 50 per cent. KOH solution to the open end and allow the mixture to stand for two days at room temperature. With certain varieties of bacteria a red color like that of eosin develops after twenty-four to forty-eight hours. With true *B. coli* this color does not develop.

CHAPTER XXXVIII.

THE BACTERIOLOGY OF MILK IN ITS RELATION TO DISEASE.

FROM the standpoint of the dairy many of the different varieties of bacteria found in milk are of importance, which have little or no medical interest. We have space here only to consider the bacteriology of milk so far as it is related to health and disease. The saprophytic bacteria taken collectively have importance because one can determine from their number something as to the care taken in handling the milk and also because, when numerous, they produce chemical changes in the milk which are harmful for infants.

Numerical Estimation of Bacteria.—The number of bacteria in a c.c. of milk is usually estimated from the colonies developing in nutrient agar plate cultures during a period of three to four days, when kept at 20° to 27° C. Some authorities prefer a temperature of 37° for 48 hours, but this allows in market milk in which bacteria have developed at low temperature only a certain proportion of the varieties of bacteria to develop colonies. Sometimes fully twice as many colonies develop at 20° to 27° C. as at 37°. For the technique see pages 43–46. This culture method necessarily underestimates their number, as many of the bacteria remain after vigorous shaking in pairs or small groups. In order to overcome this and also to note the morphological types, the direct microscopical examination of smears of the sediment has been urged. A great practical objection to this is that, if a heated milk is examined, the dead as well as the living bacteria are counted. This method has, however, great advantages in that one can immediately tell whether a sample has few or many bacteria and also note the presence of streptococci and leukocytes.

Smear Method for Direct Examination of Milk.—1. The sample of milk to be examined is shaken thoroughly, not less than twenty-five times.

2. One cubic centimeter is withdrawn and put into a tube of small calibre having two rubber corks and is centrifugalized for 10 minutes.

3. After centrifugalization the upper cork is removed and the supernatant cream and milk are gently poured off; the lower cork which holds the sediment is then removed and the sediment is spread as evenly as possible on slides in areas of two square centimetres upon which a drop of sterile water has been previously placed.

4. After drying in the air, the smears are fixed with methyl alcohol and stained with a watery solution of methylene blue. By turning the slides the excess stain drains off and washing with water is avoided with its danger of removing bacteria. The sediment contains about

33 per cent. of the bacteria in the whole milk. If there is any fat in the sediment this can be removed by flooding the slide after fixing with ½ per cent. NaOH solution.

5. Ten fields are counted in each smear, four at the top and bottom and one at either end. A net micrometer, fitted into the eyepiece and marking a field equal to $\frac{1}{10000}$ of a square centimetre is used with the oil immersion, the average counts of the ten fields are multiplied by 20,000 and the results are therefore the bacterial count of the sediment from one cubic centimetre of milk.

6. If a leukocyte count only is desired, the same technique is followed except that before centrifugalization the milk is heated to 65° to 70° C. for 10 minutes, after which it is thoroughly shaken and put into the centrifugal tubes.

Identification of Bacteria.—The milk is plated in a 2 per cent. lactose-litmus nutrient gelatin or agar, and the bacteria, after development of colonies, isolated and grown upon the usual identification media. The pathogenic properties of the different bacteria can be tested by intraperitoneal and subcutaneous inoculation in guinea-pigs with 2 c.c. of a forty-eight-hour broth culture, and by feeding young kittens for several days with 3 to 6 c.c. daily of a twenty-four-hour broth culture by means of a medicine dropper.

Varieties.—Bacteria in milk can be divided into two great groups—those which get into the milk after it leaves the udder and those which come from the cow. The first group comprises bacteria from dust, hands, milking pails, strainers, etc.

The extraneous bacteria are of importance because they produce changes in the chemical composition of the milk when they have developed in great numbers. The number of bacteria in any sample of milk depends on three factors: the number deposited in the milk from the cow's udder, from the air, and utensils; the time during which they have developed, and the temperature at which the milk has stood. The last is perhaps the most important factor. The attempt was made during a period of one year to connect illness in infants and children with special varieties of saprophytic bacteria in milk. As a matter of fact, no such connection was made out.

From the milks altogether 239 varieties of bacteria were isolated and studied. These 239 varieties, having some cultural or other differences, were divided into the 31 classes, each class containing from 1 to 39 more or less closely related organisms.

As to the sources of bacteria found in milk, we made sufficient experiments to satisfy us that they came chiefly from outside the udder and milk-ducts.

Bacteria were isolated from various materials which under certain conditions might be sources of contamination for the milk, and the cultures compared with those taken from milk. Thus there were obtained from 20 specimens of hay and grass, 31 varieties of bacteria; from 15 specimens of fæces, manure, and intestinal contents, 28 varieties; from 10 specimens of feed, 17 varieties. Of these 76

varieties there were 26 which resembled closely those from milk—viz., 11 from grass or hay; 26 from manure; 5 from feed.

During the investigation a number of the varieties isolated from milk were shown to be identical with types commonly found in water.

From the few facts quoted above and from many other observations made during the course of the work, it would seem that the term "milk bacteria" assumes a condition which does not exist in fact. The expression would seem to indicate that a few varieties, especially those derived in some way from the cow, are commonly found in milk, which forms having entered the milk while still in the udder or after its withdrawal, are so well fitted to develop in milk that they outgrow all other varieties.

As a matter of fact, it was found that milk taken from a number of cows, in which almost no outside contamination had occurred, and plated immediately, contained, as a rule, very few bacteria, and these were streptococci, staphylococci, and other varieties of bacteria not often found in milk sold in New York City; the temperature at which milk is kept being less suitable for them than for the bacteria which fall into the milk from dust, manure, etc. A number of specimens of fairly fresh market milk averaging 200,000 bacteria per cubic centimetre were examined immediately, and again after twelve to twenty-four hours. In almost every test the three or four predominant varieties of the fresher milk remained as the predominant varieties after the period mentioned.

The above experiments seem to show that organisms which have gained a good percentage in the ordinary commercial milk at time of sale will be likely to hold the same relative place for as long a period as milk is usually kept. After the bacteria pass the ten or twenty million a change occurs, since the increasing acidity inhibits the growth of some forms before it does that of others. Thus some varieties of the lactic acid bacteria can increase until the acidity is twice as great as that which inhibits the growth of many bacteria. Before milk reaches the curdling point, the bacteria may have reached over a billion to each cubic centimetre. For the most part specimens of milk from different localities showed a difference in the character of the bacteria present, in the same way that the bacteria from hay, feed, etc., varied. Even the intestinal contents of cows, the bacteriology of which might be expected to show common characteristics, contained, besides the predominating colon types, other organisms which differed widely in different species and in different localities. Cleanliness in handling the milk and the temperature at which it had been kept were also found to have a marked influence on the predominant varieties of bacteria present.

Pathogenic Properties of the Bacteria Isolated.—Intraperitoneal injection of 2 c.c. of broth or milk cultures of about 40 per cent. of the varieties tested caused death. Cultures of most of the remainder produced no apparent deleterious effects even when injected in larger amounts. The filtrates of both cultures of a number of varieties

were tested, but only one was obtained in which poisonous products were abundantly present. Death in guinea-pigs weighing 300 grams followed within fifteen minutes after an injection of 2 c.c.; 1 c.c. had little effect.

As bacteria in milk are swallowed and not injected under the skin, it seemed wise to test the effect of feeding them to very young animals. We therefore fed forty-eight cultures of 139 varieties of bacteria to kittens of two to ten days of age by means of a glass tube. The kittens received 5 to 10 c.c. daily for from three to seven days. Only one culture produced illness or death. A full report on the identification of the varieties of bacteria met with in this investigation can be found in an article by Dr. Letchworth Smith in the 1902 *Annual Report of the Department of Health of New York City.*

After five years of effort to discover some relation between special varieties of bacteria found in milk and the health of children the conclusion has been reached that neither through animal tests nor the isolation from the milk of sick infants have we been able to establish such a relation. Pasteurized or "sterilized" milk is rarely kept in New York longer than thirty-six hours, so that varieties of bacteria which after long standing develop in such milk did not enter into our problem. The harmlessness of cultures given to healthy young kittens does not of course prove that they would be equally harmless in infants. Even if harmless in robust infants, they might be injurious when summer heat and previous disease had lowered the resistance and the digestive power of the subjects.

Streptococci in Relation to Disease.—In an investigation by Dr. D. H. Bergey connection between diarrhœa and pus and streptococci was sometimes found.

The results of this investigation appear to warrant the following conclusions:

1. The occurrence of an excessive number of leukocytes in cows' milk is probably always associated with the presence in the udder of some inflammatory reaction brought about by the presence of some of the ordinary pyogenic bacteria, especially of streptococci.

2. When a cow's udder has once become infected with the pyogenic bacteria, the disease tends to persist for a long time, probably extending over several periods of lactation.

3. Lactation has no causative influence *per se* upon the cellular and bacterial content of cows' milk, though it probably tends toward the aggravation of the disease when the udder is once infected.

It is impossible to differentiate in routine milk examinations the pathogenic streptococci of diseased cows from saprophytic varieties. Thus it happens that a milk which contains great numbers of streptococci may or may not be more dangerous than one which contains an equal number of other apparently less harmful bacteria.

The Deleterious Effect of Bacteria in Milk on Infants.—We have tested this ourselves in the following way: During each of the summers of 1902, 1903, and 1904 a special lot of milk was modified for

a group of fifty infants, all of whom were under nine months of age, and distributed daily. To one half the milk was given raw; to the other half a similar milk heated at 60° for 20 minutes.

The modified milk was made from a fairly pure milk mixed with ordinary cream. The bacteria contained in the milk numbered on the average 45,000 per cubic centimetre, in the cream 30,000,000. The modified raw milk taken from the bottles in the morning averaged 1,200,000 bacteria per cubic centimetre, or considerably less than the ordinary grocery milk; the Pasteurized, about 1000; taken in the late afternoon of the same day they had, respectively, about 20,000,000 and 50,000.

Twenty-one predominant varieties of bacteria were isolated from six specimens of this milk collected on different days. The varieties represented the types of bacteria frequently found in milk. The infants were selected during the first week in June, and at first all were placed on Pasteurized milk. The fifty infants which had been selected were now separated into two groups as nearly alike as possible. On the 15th of June the milk was distributed without heating to one half the infants, the other half receiving as before the heated milk. In this way the infants in the two groups received milk of identically the same quality, except for the changes produced by heating to 165° F. for thirty minutes. The infants were observed carefully for three months and medical advice was given when necessary. When severe diarrhœa occurred barley-water was substituted for milk.

The first season's trial gave the following results: Within one week 20 out of the 27 infants put on raw milk suffered from moderate or severe diarrhœa; while during the same time only 5 cases of moderate and none of severe diarrhœa occurred in those taking Pasteurized milk. Within a month 8 of the 27 had to be changed from raw back to heated milk, because of their continued illness; 7, or 25 per cent., did well all summer on raw milk. On the other hand, of those receiving the Pasteurized milk, 75 per cent. remained well, or nearly so, all summer, while 25 per cent. had one or more attacks of severe diarrhœa. There were no deaths in either group of cases.

During the second summer a similar test was made with 45 infants. Twenty-four were put on raw modified milk; 13 of these had serious diarrhœa, in 5 of whom it was so severe that they were put back upon heated milk; 10 took raw milk all summer without bad effects; 2 died, 1 from gross neglect on the part of the mother, the other from diarrhœa. Of the 21 on Pasteurized milk, 5 had severe attacks of diarrhœa, but all were kept on this milk except for short periods, when all food was omitted; 16 did well throughout the summer. One infant, markedly rachitic, died. The third summer's results have not been tabulated, but were similar to those of the first two tests.

The outcome of these observations during the first two summers are summarized in the following table:

Kinds of milk	Number of infants	Remained well for entire summer	Number having severe or moderate diarrhœa	Average number days off milk during summer	Average weekly gain in weight	Average number of days diarrhœa	Deaths
Pasteurized milk, 1,000 to 50,000 bacteria per c.c.	41	31	10	3	4.0 oz.	3.9	1
Raw milk, 1,200,000 to 20,000,000 bacteria per c.c.	51[1]	17	33	5.5	3.5 oz.	11.5	2

Although the number of cases was not large, the results, almost identical during the three summers, indicate that even a fairly pure milk, when given raw in hot weather, causes illness in a much larger percentage of cases than the same milk given after Pasteurization. A considerable percentage of infants, however, do apparently quite as well on raw as on Pasteurized milk.

Bacteria in Milk. Effect on Older Children—The children over three years of age who received unheated milk, containing at different times from 145,000 to 350,000,000 bacteria per cubic centimetre, showed almost no gastrointestinal disturbance. The conditions at three institutions will serve as examples.

In the first of these an average grade of raw milk was used which, during the summer, contained from 2,000,000 to 30,000,000 bacteria per cubic centimetre. This milk was stored in an ice-box until required. It was taken by children unheated and yet no case of diarrhœa of sufficient gravity to send for a physician occurred during the entire summer. This institution was an orphan asylum containing 650 children from three to fourteen years of age—viz., three to five years, 98; five to eight years, 162; eight to fourteen years, 390.

A second institution used an unheated but very pure milk which was obtained from its own farm. This milk averaged 50,000 bacteria per cubic centimetre. The inmates were 70 children of ages ranging from three to fourteen years. In this institution not a single case of diarrhœal disease of any importance occurred during the summer.

In a third institution an average grade of milk was used which was heated. This milk before heating contained 2,000,000 to 20,000,000 bacteria per cubic centimetre. The institution was an infant asylum in which there were 126 children between the ages of two and five years. There were no cases of diarrhœa during the summer.

These clinical observations taken in connection with the bacteriological examination at the laboratory show that although the milk may come from healthy cattle and clean farms and be kept at a temperature not exceeding 60° F., a very great increase in the number

[1] Thirteen of the fifty-one infants on raw milk were transferred before the end of the trial to Pasteurized milk because of serious illness. If these infants had been left on raw milk it is believed by the writers that the comparative results would have been even more unfavorable to raw milk.

of bacteria may occur. Furthermore, this may occur without the accumulation in the milk of sufficient poisonous products or living bacteria to cause appreciable injury in children over three years of age, even when such milk is consumed in considerable amount and for a period extending over several months. Milk kept at temperatures somewhat above 60° F. was not met with in our investigations, but the histories of epidemics of ptomain poisoning teach that such milk may be very poisonous. It is also to be remembered that milk abounding in bacteria on account of its being carelessly handled is also always liable to contain pathogenic organisms derived from human or animal sources.

Results with Very Impure Milk Heated vs. Those with Pure or Average Milk Heated.—During the summer of 1901 we were able to observe a number of babies fed on milk grossly contaminated by bacteria. In 1902 systematic supervision of all stores selling milk was instituted by the Health Department, so that the very worst milk was not offered for sale that summer.

The observations upon the impure milk of 1901 are of sufficient importance to be given in detail, although already mentioned in the report of the observations upon infants of both summers which were fed on "store milk." A group of over 150 infants was so divided that 20 per cent. were allowed to remain on the cheapest store milk which they were taking at the time. To about the same number was given a pure bottled milk. A third group was fed on the same quality of milk as the second, but sterilized and modified at the Good Samaritan Dispensary. A fourth group received milk from an ordinary dairy farm. This milk was sent to a store in cans and called for by the people. A few infants fed on breast and condensed milk were observed for control.

In estimating the significance of the observations recorded in the tables, one should bear in mind that not only do different infants possess different degrees of resistance to disease, but that, try as hard as the physicians could, it was impossible to divide the infants into groups which secured equal care and were subjected to exactly the same conditions. It was necessary to have the different groups in somewhat different parts of the city. It thus happened that the infants on the cheap store milk received less home care than the average, and that those on the pure bottled milk lived in the coolest portion of the city. Certain results were, however, so striking that their interpretation is fairly clear. It is to be noted that the number of infants included in each group is small.

There is nothing in the observations to show that fairly fresh milk from healthy cows, living under good hygienic conditions and containing, on some days, when delivered, as many as 200,000 bacteria per cubic centimetre, had any bacteria or any products due to bacteria that remained deleterious after the milk was heated to near the boiling point.

On the other hand, it is possible that certain varieties of bacteria

TABLE SHOWING THE RESULTS OF FEEDING DURING JULY AND AUGUST, 1901, IN TENEMENT HOUSES, OF 112 BOTTLE-FED INFANTS UNDER 1 YEAR OF AGE, AND OF 47 BOTTLE-FED INFANTS BETWEEN 1 AND 2 YEARS OF AGE WITH MILK FROM DIFFERENT SOURCES, AND THE NUMBER OF BACTERIA PRESENT IN THE MILK.

Character of the milk	Infants under one year					Infants over one year				
	Number of infants	Average weekly gain	Diarrhœa		Deaths	Number of infants	Average weekly gain	Diarrhœa		Deaths
			Mild	Severe				Mild	Severe	
1. Pure milk boiled and modified at dispensary or stations; given out in small bottles. Milk before boiling averaged 20,000 bacteria per c.c.; after boiling 2 per c.c.	41	3 oz.	10	8	1[1]					
2. Pure milk, 24 hours old, sent in quart bottles to tenements, heated and modified at home, 20,000 to 200,000 bacteria per c.c. when delivered.	23	4½ oz.	8	5	0	24	4½ oz.	8	2	0
3. Ordinary milk, 36 hours old, from a selected group of farms, kept cool in cans during transport; 1,000,000 to 25,000,000 bacteria per c.c., heated and modified at home before using.	18	4 oz.	6	6	1[2]	12	4 oz.	1	2	0
4. Cheap milk, 36 to 60 hours old, from various small stores, derived from various farms, some fairly clean, some very dirty; 400,000 to 175,000,000 bacteria per c.c.	21	½ oz.	4	13	4[3]	7	½ oz.	1	3	0
5. Condensed milk of different brands. Made up with hot water. As given, contained bacteria from 5,000 to 200,000 per c.c.	9	½ oz.	5	2	3	4	3½ oz.	1	3	0
6. Breast milk	16	2½ oz.	5	2	0					

may, under conditions that are unsanitary, find entrance to milk and survive moderate heat or may develop poisonous products resistant to heat in sufficient amount to be harmful, even when they have accumulated to less than 200,000 per cubic centimetre.

Turning now to the results of feeding with milk which has been heated and which before sterilization contained from 1,000,000 to 25,000,000 bacteria per cubic centimetre, averaging about 15,000,000, though obtained from healthy cows living under fairly decent conditions and although the milk was kept moderately cool in transit, we find a distinct increase in the amount of diarrhœal diseases.

[1] This infant died from enteritis and toxæmia.

[2] This infant died of pneumonia. There had been no severe intestinal disorder noted.

[3] One of the four had pertussis, the remaining three died from uncomplicated enteritis.

Though it is probable that the excessive amount of diarrhœa in this group of children was due to bacterial changes which were not neutralized by heat or to living bacteria which were not killed, yet it is only fair to consider that the difference was not very great and that the infants of this group were under surroundings not quite as good at those on the purer milk.

Finally, we come in this comparison to the infants who received the cheap store milk after heating. This milk had frequently to be returned because it curdled when boiled, and contained, according to the weather, from 4,000,000 to 200,000,000 bacteria per cubic centimetre. In these infants the worst results were seen. This is shown not only by the death rate, but by the amount and by the severity of the diarrhœal diseases, and the general appearance of the children as noted by the physicians. Although the average number of bacteria in the milk received by this group is higher than that received by the previous group, the difference in results between this group and the previous one can hardly be explained by the difference in the number of bacteria. The varieties of bacteria met with in this milk were more numerous than in the better milk, but we were unable to prove that they were more dangerous. Probably the higher temperature at which the milk was kept in transit, and the longer interval between milking and its use, allowed more toxic bacterial products to accumulate.

Bacterial Contamination of Milk—General Conclusions[1] as to Relative Importance.—1. During cool weather neither the mortality nor the health of the infants observed in the investigation was appreciably affected by quality of the market milk or by the number of bacteria which it contained. The different grades of milk varied much less in the amount of bacterial contamination in winter than in summer, the store milk averaging only about 750,000 bacteria per cubic centimetre.

2. During hot weather, when the resistance of the children was lowered, the kind of milk taken influenced both the amount of illness and the mortality; those who took condensed milk and cheap store milk did the worst, and those who received breast milk, pure bottled milk, and modified milk did the best. The effect of bacterial contamination was very marked when the milk was taken without previous heating; but, unless the contamination was very excessive, only slight when heating was employed shortly before feeding.

3. The number of bacteria which may accumulate before milk becomes noticeably harmful to the average infant in summer differs with the nature of the bacteria present, the age of the milk, and the temperature at which it has been kept. When the milk is taken raw, the fewer the bacteria present the better are the results. Of the usual varieties, over 1,000,000 bacteria per cubic centimetre are certainly deleterious to the average infant. However, many infants

[1] These conclusions were drawn up by the writer in association with Dr. L. E. Holt, after a joint study of the results obtained in the studies above recorded.

take such milk without apparently harmful results. Heat above 170° F. (77° C.) not only destroys most of the bacteria present, but, apparently, some of their poisonous products. No harm from the bacteria previously existing in recently heated milk was noticed in these observations unless they had amounted to many millions, but in such numbers they were decidedly deleterious.

4. When milk of average quality was fed, sterilized and raw, those infants who received milk previously heated did, on the average, much better in warm weather than those who received it raw. The difference was so quickly manifest and so marked that there could be no mistaking the meaning of the results.

5. No special varieties of bacteria were found in unheated milk which seemed to have any special importance in relation to the summer diarrhœas of children. A few cases of acute indigestion were seen immediately following the use of Pasteurized milk more than thirty-six hours old. Samples of such milk were found to contain more than 100,000,000 bacteria per cubic centimetre, mostly spore-bearing varieties. The deleterious effects, though striking, were neither serious nor lasting.

6. After the first twelve months of life, infants are less and less affected by the bacteria in milk derived from healthy cattle. According to these observations, when the milk had been kept cool, the bacteria did not appear to injure the children over three years of age at any season of the year, unless in very great excess.

7. Since a large part of the tenement population must purchase its milk from small dealers, at a low price, everything possible should be done by health boards to improve the character of the general milk supply of cities by enforcing proper legal restrictions regarding its transportation, delivery, and sale. Sufficient improvements in this respect are entirely feasible in every large city to secure to all a milk which will be wholesome after heating. The general practice of heating milk, which has now become a custom among the tenement population of New York, is undoubtedly a large factor in the lessened infant mortality during the hot months.

8. Of the methods of feeding now in vogue, that by milk from central distributing stations unquestionably possesses the most advantages, in that it secures some constant oversight of the child, and, since it furnishes the food in such a form that it leaves the mother least to do, it gives her the smallest opportunity of going wrong. This method of feeding is one which deserves to be much more extensively employed, and might, in the absence of private philanthropy, wisely be undertaken by municipalities and continued for the four months from May 15th to September 15th.

9. The use, for infants, of milk delivered in sealed bottles, should be encouraged whenever this is possible, and its advantage duly explained. Only the purest milk should be taken raw, especially in summer.

10. Since what is needed most is intelligent care, all possible means

should be employed to educate mothers and those caring for infants in proper methods. This, it is believed, can most effectively be done by the visits of properly qualified trained nurses or women physicians to the homes, supplemented by the use of printed directions.

11. Bad surroundings, though contributing to bad results in feeding, are not the chief factors. It is not, therefore, merely by better housing of the poor in large cities that we will see a great reduction in infant mortality.

12. While it is true that even in tenements the results with the best bottle feeding are nearly as good as average breast feeding, it is also true that most of the bottle feeding is at present very badly done; so that, as a rule, the immense superiority of breast feeding obtains. This should, therefore, be encouraged by every means, and not discontinued without good and sufficient reasons. The time and money required for artificial feeding, if expended by the tenement mother to secure better food and more rest for herself, would often enable her to continue nursing with advantage to her child.

13. The injurious effects of table food to infants under a year old, and of fruits to all infants and young children in cities, in hot weather, should be much more generally appreciated.

Influence of Temperature upon the Multiplication of Bacteria in Milk.—Few, even of the well informed, appreciate how great a difference a few degrees of temperature will make in the rate of bacterial multiplication. Milk rapidly and sufficiently cooled keeps almost unaltered for thirty-six hours, while milk insufficiently cooled deteriorates rapidly.

The majority of the bacteria met with in milk grow best at temperatures above 70° F., but they also multiply slowly even at 40° F.; thus of 60 species isolated by us, 42 developed good growths at the end of seven days at 39° F. Our observations have shown that the bacteria slowly increase in numbers after the germicidal properties of the milk have disappeared, and the germs have become accustomed to the low temperature. In fact, milk cannot be permanently preserved unaltered unless kept at 32° F. or less. The degree of cooling to which ordinary supplies of milk are subjected differs greatly in various localities. Some farmers chill their milk rapidly, by means of pipe coils over which the milk flows; others use deep wooden tanks filled with water into which the cans of milk are placed soon after milking. In winter these methods are very satisfactory for the water runs into the pipes or tanks at about 38° F. In warmer weather they are unsatisfactory, unless ice is used, as the natural temperature of the water may be as high as 55° F. A considerable quantity of milk is not cooled at all at the farms. It is sent to the creamery or railroad after two to six hours, and is then more or less cooled. These few hours in summer, when the milk is left almost at blood heat, allow an enormous development of bacteria to take place, as is shown in the table below.

TABLE I.—Showing the development of bacteria in two samples of milk maintained at different temperatures for twenty-four, forty-eight, and ninety-six hours, respectively. The first sample of milk was obtained under the best conditions possible, the second in the usual way. When received, specimen No. 1 contained 3000 bacteria per c.c., specimen No. 2, 30,000 per c.c.

Temperature. Fahrenheit.	Time which elapsed before making test. 24 hrs.	48 hrs.	96 hrs.	168 hrs.
32°	2400	2100	1850	1400
	30,000[1]	**27,000**	**24,000**	**19,000**
39°	2500	3600	218,000	4,209,000
	38,000	**56,000**	**4,300,000**	**38,000,000**
42°	2600	3600	500,000	11,200,000
	43,000	**210,000**	**5,760,000**	**120,000,000**
46°	3100	12,000	1,480,000	80,000,000
	42,000	**360,000**	**12,200,000**	**300,000,000**
50°	11,600	540,000	300,000,000	1,000,000,000[2]
	89,000	**1,940,000**	**1,000,000,000**[2]	
55°	18,800	3,400,000		
	187,000	**38,000,000**		
60°	180,000	28,000,000		
	900,000	**168,000,000**		
68°	450,000	500,000,000		
	4,000,000	**1,000,000,000**[2]		
86°	1,400,000,000[2]			
	14,000,000,000[2]			

Observations on Bacterial Multiplication in Milk at 90° F., a Temperature Common in New York in Hot Summer Weather.

TABLE II.—*Number of Bacteria per 1 c.c.*

	Milk I. Fresh and of good quality	Milk II. Fair quality from store.	Milk III. Bad quality from store.
Original number	5200	92,000	2,600,000
After two hours	8400	184,000	4,220,000
After four hours	12,400	470,000	19,000,000
After six hours	68,500	1,260,000	39,000,000
After eight hours	654,000	6,800,000	124,000,000

A sample of milk No. I. removed after six hours and cooled to 50° F. contained 145,000,000 at the end of twenty-four hours. Some of this milk, kept cool from the beginning, contained but 12,800 bacteria per c.c. at the end of twenty-four hours.

Pasteurization of Milk.—The two dominant factors which control the temperature and time at which the milk should be heated are (1) the thermal death points of pathogenic bacteria, and (2) the thermolabile food constituents of the milk. The first factor is almost equally important for milk used by persons of all ages, while the second factor is only important for milk used in very young children.

The exposure of bacteria for a short time at a high temperature is equivalent to a longer time at a lower temperature. The ferments and other labile food constituents, on the other hand, are altered much more by the higher temperature. It is well, therefore, to choose the lowest possible temperature which will kill the non-spore-bearing patho-

[1] The figures referring to tests of the second sample are printed in heavy-face type.

[2] These figures are fairly accurate estimates.

genic bacteria in a practicable length of time. Such an exposure is 60° C. (140° F.) for 20 minutes or 70° C. 158° F.) for 5 minutes. Very much shorter exposures, as one minute at 70° C., will kill the great majority of pathogenic and other bacteria in the milk and add much of safety as seen in the tables below, but it is better to be on the safe side.

Table showing effect of heat upon tubercle bacilli in milk heated instantly.

Degree of heat.	Time exposed.	Amount milk.	Result in guinea-pigs.
60° C.	15 min.	1 c.c.	Infection
60° C.	20 min.	1 c.c.	No infection
60° C.	30 min.	1 c.c.	No infection
70° C.	0.5[1] min.	1 c.c.	Infection[1]
70° C.	1 min.	1 c.c.	No infection
70° C.	2 min.	1 c.c.	No infection
Control not heated		.001	Infection

[1] This milk was infected by adding one-fifth of its quantity of sputum rich in tubercle bacilli.

Development of Bacteria in Heated Milk.—There is a common idea that bacteria develop much more rapidly in milk that has been heated than in raw milk. This is only true for freshly drawn milk which has slight bactericidal power.

The table below shows the effect on bacteria in milk of heating to 70° C. for one-half and one minute. Not only the immediate reduction in number is seen to be great, but the difference continues when the milk is kept cold for two days.

Two samples mixed from 100 samples of inspectors. Pasteurized at 160° F. Plates made same day.

Sample I.		Sample II.	
Control	600,000	Control	5,400,000
½ m	2000	½ m	7400
1 m	1000	1 m	600
Same Samples Kept in Ice-box 24 hrs. at 45° F. (7° C.).			
Control	6,300,000	Control	21,600,000
½ m	18,000	½ m	12,000
1 m	900	1 m	3600
In Ice-box 48 hrs. at 45° F. (7° C.).			
Control	16,200,000	Control	63,000,000
½ m	120,000	½ m	276,000
1 m	10,000	1 m	90,000
In Room at 71° F. (22° C.).			
Control	36,600,000	Control	150,000,000
½ m	5,460,000	½ m	4,500,000
1 m	5,400,000	1 m	3,600,000

Number of Bacteria in Milk Produced under Different Conditions.

1. The number of bacteria present at the time of milking and twenty-four, forty-eight, and seventy-two hours afterward in milk obtained and kept under correct conditions.

No preservatives were present in any of the following specimens:

Pure milk obtained where every reasonable means was taken to ensure cleanliness. The long hairs on the udder were clipped; the cows roughly cleaned and placed in clean barns before milking; the udders were wiped off just previous to milking; the hands of the men were washed and dried; the pails used had small (six-inch) openings, and were thoroughly cleaned and sterilized by steam before use. Milk cooled within one hour after milking to 45° F., and subsequently kept at that temperature. The first six specimens were obtained from individual cows; the last six from mixed milk as it flowed at different times from the cooler. Temperature of barns 55° F.

[1] Most of the guinea-pigs were not infected by the milk heated for one-half minute.

Number of Bacteria in 1 *c.c. of Milk.*[1]

From six individual cows.

	5 hrs. after milking.	After 24 hrs.	After 48 hrs.	After 72 hrs.
	500	700	12,500	Not counted.
	700	700	29,400	Not counted.
	19,900	5200	24,200	Not counted.
	400	200	8600	Not counted.
	900	1600	12,700	Not counted.
	13,600	3200	19,500	Not counted.
Average	6000	1933	17,816	

From mixed milk of entire herd.

	6900	12,000	19,800	494,000
	6100	2200	20,200	550,000
	4100	700	7900	361,000
	1200	400	7100	355,000
	6000	900	9800	445,000
	1700	400	8700	389,000
Average	4333	2766	10,583	329,000

Twenty-five samples taken separately from individual cows on another day and tested immediately averaged 4550 bacteria per c.c. and 4500 after twenty-four hours. These twenty-five specimens were kept at between 45° and 50° F.

2. Milk taken during winter in well-ventilated, fairly clean, but dusty barns. Visible dirt was cleaned off the hair about the udder before milking. Milkers' hands were wiped off, but not washed. Milk pails and cans were clean, but the straining cloths dusty. Milk cooled within two hours after milking to 45° F.

Number of Bacteria in 1 *c.c. of Milk.*

	At time of milking.	After 24 hrs.	After 48 hrs.
	12,000	14,000	57,000
	13,000	20,000	65,000
	21,500	31,000	106,000
Average	15,500	21,666	76,000

Number in City Milk.

3. The condition of the average city milk is very different, and is shown in the following tables.

The twenty samples were taken late in March by Inspectors of the Department of Health of New York City from cans of milk immediately upon their arrival in the city.

The temperature of the atmosphere averaged 50° F. during the previous twenty-four hours. The temperature of the milk when taken from the cans averaged 45° F. Much of this milk had been carried over two hundred miles. From the time of its removal from the cans, which was about 2 A. M., until its dilution in nutrient agar, at 10 A. M., the milk was kept at about 45° F.

[1] Number of bacteria obtained from development of colonies in nutrient agar in Petri plates. The nutrient medium contained 2 per cent. peptone and 1.2 per cent. agar, and was faintly alkaline to litmus. One set of plates were usually left four days at about 20° C., and one set twenty-four hours at 37° C., and then twenty-four hours at 20° C. From 5 to 300 per cent. more colonies developed, as a rule, in the plates kept at room temperature than in those kept for twenty-four hours at 37° C. The milk was diluted as desired with 100 or 10,000 parts of sterile water, and 1 c.c. of the diluted milk was added to 8 c.c. of melted nutrient agar. Plates containing over 1000 colonies were found to be inaccurate, in that they gave too low totals. Apparently a considerable number of bacteria failed to develop colonies when too many were added to the nutrient agar. Nutrient gelatin was found to be more troublesome and not to yield more accurate results than nutrient agar.

From New York and Hudson River Railroad.		*From Harlem Railroad.*	
No. of sample.	No. of bacteria in 1 c.c.	No. of sample.	No. of bacteria in 1 c.c.
50	35,200,000	48	6,200,000
51	13,000,000	49	2,200,000
52	2,500,000	50	15,000,000
53	1,400,000	51	70,000
54	200,000	52	80,000
55	600,000	53	320,000

While the above figures indicate that much of the milk sold is fair, even in summer, they show an appalling condition for most of that sold to the poorer classes—those who not only comprise the larger part of the population, but who are also compelled to keep their children in town during the hot weather.

It must be kept in mind that milk averaging 3,000,000 bacteria per cubic centimetre will, when kept at the temperature common in the homes of the poor, soon contain very largely increased numbers and show its dangerous condition by turning sour and curdling.

Cleanliness Used in Obtaining Milk, and Its Influence.—The present conditions under which much of the milk is obtained are not pleasant to consider. In winter, and to a less extent at other seasons of the year, the cows in many stables stand or lie down in stalls in the rear portion of which there is from one to four inches of manure and urine. When milked the hands of the milkers are not cleansed, nor are the under portions of the cows, only visible masses of manure adhering to the hair about the udder being removed. Some milkers even moisten their hands with milk, to lessen friction, and thus wash off the dirt of their hands and the cow's teats into the milk in the pails. Some may regard it as an unnecessary refinement to ask that farmers should roughly clean the floors of their stalls once each day, that no sweeping should be done just before milking, and that the udders should be wiped with a clean damp cloth and the milkers should thoroughly wash and wipe their hands before commencing milking. The pails and cans should not only be carefully cleansed, but afterward scalded out with boiling water. The washing of the hands would lessen the number of ordinary filth bacteria in the milk, and diminish risk of transmitting to milk human infectious diseases, like scarlet fever, diphtheria, and enteric fever, by the direct washing off of the disease germs from infected hands. It would also inculcate general ideas of the necessity of cleanliness and of the danger of transmitting disease through milk. The value of cleanliness in limiting the number of bacteria is demonstrated by the figures contained in the tables.

General Conclusions.—Because of its location and its hairy covering, the cow's udder is always more or less soiled with dirt and manure unless cleaned. On account of the position of the pail and the access of dust-laden air it is impossible to obtain milk by the usual methods without mingling with it a considerable number of bacteria. With suitable cleanliness, however, the number is far less than when filthy

methods are used, there being no reason why fresh milk should contain in each cubic centimetre, on the average, more than 12,000 bacteria per c.c. in warm weather and 5000 in cold weather. Such milk, if quickly cooled to 46° F., and kept at that temperature, will at the end of thirty-six hours contain on the average less than 50,000 bacteria per cubic centimetre, and if cooled to 40° F. will average less than its original number.

With only moderate cleanliness such as can be employed by any farmer without adding appreciably to his expense, namely, clean pails, straining cloths, cans or bottles, and hands, a fairly clean place for milking, and a decent condition of the cow's udder and the adjacent belly, milk when first drawn will not average in hot weather over 30,000, and in cold weather not over 25,000 bacteria per cubic centimetre. Such milk, if cooled and kept at 50° F., will not contain at the end of twenty-four hours over 100,000 bacteria per cubic centimetre. If kept at 40° F. the number of bacteria will not be over 100,000 per cubic centimetre after forty-eight hours.

If, however, the hands, cattle, and barns are filthy and the pails are not clean, the milk obtained under these conditions will, when taken from the pail, contain very large numbers of bacteria, even up to a million or more per cubic centimetre.

Freshly drawn milk contains a slight and variable amount of bactericidal substances which are capable of inhibiting bacterial growth. At temperatures under 50° F. these substances act efficiently (unless the milk is filthy) for from twelve to twenty-four hours, but at higher temperatures their effect is very soon completely exhausted, and the bacteria in such milk will then rapidly increase. Thus the bacteria in fresh milk which originally numbered 5000 per cubic centimetre decreased to 2400 in the portion kept at 42° F. for twenty-four hours, but rose to 7000 in that kept at 50° F., to 280,000 in that kept at 65° F., and to 12,500,000,000 in the portion kept at 95° F.

As we have seen, the milk in New York City is found on bacteriological examination to contain, as a rule, excessive numbers of bacteria. During the coldest weather the milk in the shops averages over 300,000 bacteria per cubic centimetre, during cool weather about 1,000,000, and during hot weather about 2,000,000. The milk in other large cities is, from all accounts, in about the same condition.

The above statement holds for milk sold at the ordinary shops, and not that of the best of the special dairies, where, as previously stated, the milk contains only from 1000 to 30,000 bacteria, according to the season of the year.

The question might be raised, Are even these enormous numbers of bacteria often found in milk during hot weather harmful?

Our knowledge is probably as yet insufficient to state just how many bacteria must accumulate to make them noticeably dangerous in milk. Some varieties are undoubtedly more harmful than others, and we have no way of restricting the kinds that will fall into milk,

except by enforcing cleanliness. We have also to consider that milk is not entirely used for some twelve hours after being purchased, and that during all this time bacteria are rapidly multiplying, especially where, as among the poor, no provision for cooling it is made. Slight changes in the milk which to one child would be harmless, would in another produce disturbances which might lead to serious disease. A safe conclusion is that no more bacterial contamination should be allowed than it is practicable to avoid. Any intelligent farmer can use sufficient cleanliness and apply sufficient cold, with almost no increase in expense, ½ cent per quart, to supply milk twenty-four to thirty-six hours old which will not contain in each cubic centimetre over 50,000 to 100,000 bacteria, and no milk containing more bacteria should be sold.

The most deleterious changes which occur in milk during its transportation are now known not to be due to skimming off the cream or to the addition of water, but to the changes produced in the milk by multiplication of bacteria. During this multiplication, acids and distinctly poisonous bacterial products are added to the milk, to such an extent that much of it has become distinctly deleterious to infants and invalids It is the duty of health authorities to prevent the sale of milk rendered unfit for use through excessive numbers of bacteria and their products.

The culture tests to determine the number of bacteria present in any sample of milk require at least forty-eight hours; so that the sale of milk found impure cannot be prevented. It will, however, be the purpose of the authorities gradually to force the farmers and the middlemen to use cleanliness, cold, and dispatch in the handling of their milk, rather than to prevent the use of the small amount tested on any one day.

If the milk on the train or at the dealer's were found to contain excessive numbers of bacteria, the farmers would be cautioned and instructed to carry out the simple necessary rules, which would be furnished.

Transmisson of Contagious Diseases through Milk.—Pathogenic Bacteria in Milk.—Tuberculosis, typhoid fever, scarlet fever, diphtheria and tonsillitis are the chief diseases transmitted by means of milk in this locality. In other countries cholera, malta fever and possibly other diseases may be due at times to milk infection. The obscure disease trembles is also believed to be due to milk.

The tubercle bacilli are in the majority of cases derived from the cow, but may come from human sources, the typhoid bacilli are entirely from man, the contagion of true scarlet fever conveyed in milk is probably always from man, but the contagion of a disease closely allied to it is certainly given off by cows suffering from certain septic diseases as yet not fully identified. Diphtheria bacilli are probably always of human origin, as animals, except cats, practically never suffer from the disease and these only under exceptional conditions. The streptococci exciting tonsillitis are probably usually from cases of septic inflammation of the udder, but possibly may at times come from man. As milk is usually

kept below 60° F. the typhoid bacilli and the streptococci are the only germs that we believe increase in any appreciable extent.

The following epidemics and cases have been recorded in the bulletin of the Marine Hospital service, as produced by cow's milk:

	Epidemics.	Cases.
Typhoid fever	179	6900
Scarlet fever	51	2400
Sore throat	7	1100
Diphtheria	23	960
Tuberculosis	..	..

The cases of trembles (milk sickness), believed to be due to milk, have not been collected with sufficient care to be reported. No case of measles, smallpox, whooping cough, or mumps has been clearly traced to milk.

The Relation of the Typhoid Carrier to Milk Infection.—Many epidemics of typhoid fever have until recently puzzled investigators because, though evidently milk-borne, yet no case of typhoid fever could be found. The discovery that about 2 per cent. of those who have recovered from typhoid fever remain infected and continue during the rest of their lives to pass typhoid bacilli has cleared up the mystery. Epidemics due to these carriers have already been traced both in New-York City and elsewhere. Many observers have already discussed the relation of typhoid cases to milk infection. Hands, water, flies, etc., may all aid in the transfer of the bacilli from the dejecta to the milk. Last year we traced over four hundred cases to infection of a milk supply by a typhoid carrier who had the disease forty-seven years ago. Just recently we traced fifty cases to a man who had the disease seven years ago.

The Conveyance of Scarlet Fever by Means of Milk.—As we do not know the organism which excites scarlet fever, we are not as clear as to the means by which it is spread as we are in the case of tuberculosis, typhoid fever and diphtheria. We know, however, that the throat secretions and the peeling scales of skin are dangerous. Where the infection has been traced it has usually been found that the milker has suffered from an unrecognized case or is convalescent. It seems as if the contagion must either increase in milk or be capable of infecting when greatly diluted, for cases have developed from milk after great dilution. A small number of epidemics have appeared to come from the milk of diseased cows. Many are skeptical about this, but after personal experience we cannot doubt it. The history of this case was as follows: The milk from a septic cow was delivered to two schools. About thirty of the boys who drank the milk developed the disease while none of the day scholars who went home to lunch did. Some of the cases developed at first only sore throats, others only the rash. On the second day the cases resembled very closely scarlet fever. There was no scarlet fever in the town. The milk contained immense numbers of long-chained streptococci.

Diphtheria and septic sore throats are occasionally produced by milk. The diphtheria bacilli usually originate from a mild case, the nature of which is not detected. Septic sore throats produced by milk are usually caused by infection from cows suffering from some form of udder disease.

PART III.
PROTOZOA

CHAPTER XXXIX.

GENERAL CHARACTERISTICS AND CLASSIFICATION.

Introduction.—Recent discoveries relating to the origin of human diseases are adding to the number caused, or probably caused by Protozoa. Indeed, the fact that the specific etiological factor in malaria is a protozoön, has not been known long, though this organism is the first protozoön shown to be pathogenic for man. The evidence which has been accumulating in favor of the idea that a certain form of dysentery is due to an ameba is gaining ground; quite recently sleeping sickness and kala-azar have been added to the list of protozoan diseases, and it is now thought that some of the members of the group of contagious diseases, known as the exanthemata, may be due to infection with organisms belonging to this sub-kingdom; therefore it becomes more and more necessary for those interested in the etiology, course, and prevention of disease to obtain a more definite understanding of this great group of microörganisms.

In any treatise on pathogenic organisms which is intended to aid the medical student and practitioner special attention should be given to the effect of the organism upon the host and to methods of diagnosis and treatment. Therefore symptoms of the disease, tissue changes in the host, and special staining and other methods for diagnosing the organisms are described. Only those characteristics of the organisms are given which will help in recognizing them in disease. For minutia of morphology, theories in regard to relationship, and other special points relating to the organisms themselves the student should consult such books as Calkins' "Protozoölogy."

Definition.—A protozoön (the lowest form of animal life) is a morphologically single-celled organism, composed of protoplasm which is differentiated into cytoplasm and nucleus (or nuclear substance), both of which show many variations throughout the more or less complicated life cycle that each individual undergoes.

Relationship to Other Microörganisms.—They are classed as the lowest animals, but they are so closely related to the protophyta or lowest plant forms on one side and the metazoa or many-celled animals on the other, that it is difficult to mark out a sharp line of distinction on either side. Following Haeckel, some authors group them with bacteria and other closely related forms as protista, but in such a group they should be regarded as of a

higher grade than bacteria because of their greater complexity in structure and life cycle.[1]

Whether one of the simplest microörganisms is a plant or an animal is often difficult to decide, hence there are a number of forms which are claimed by both botanists and zoölogists. For example the *Mycetozoa* are described by both, and some members of the group are contested for by each. Again it is not yet decided whether the spirochetes belong to the bacteria or to the protozoa.

The difficulty in deciding as to the plant or animal nature of these low organisms is due to the fact that the obvious differences which exist between a higher plant and a higher animal are not seen here. There is no one distinctive characteristic which separates the lowest plants from the lowest animals. In the broad sense, vegetal nutrition is the using of more simple nitrogenous substances than the proteids or peptones needed by animals, as well as the mineral substances and the organic carbon compounds required to build up their protoplasm. But if we classify organisms according to their morphology, we find that many forms placed with the *bacteria* require complex specially prepared food similar to that needed by animals; likewise, if we depend upon a physiologic distinction, we find that chlorophyl which is supposed to be a characteristic plant substance is possessed both by some bacteria and by some protozoa; and so on, through the whole list of supposed differential characteristics. Even when we do the better thing and make a third kingdom, the *protista*, some doubtful forms will always be found on the border line.

Historical Notes.[2]—The history of protozoa begins with that of bacteria in the discoveries of A. Van Leeuwenhoeck and his followers during the latter part of the seventeenth century.[3] At that time all of the microscopic organisms seen were classed together as little animals. Indeed, all of the microörganisms first described at any length were probably protozoa and only after further improvement of lenses and a more minute study of the organisms were bacterial forms gradually recognized as a separate class.

The same scepticism that is seen in the acceptance of most new discoveries was displayed by doubters of the truth of these early reports of microscopic findings. Chief among the sceptics must be placed Linnæus who in the first edition of his *Systema Naturæ* (1735) absolutely denies the existence of Leeuwenhoeck's animalcula, though in the later editions he grudgingly admits them under the significant generic name of *Chaos* (*Chaos proteus* (*Amœba*), etc.).

The first ideas of the structure of the protozoa were drawn from analogy. The early observers thought that each tiny organism possessed an internal structure made up of organs and tissues similar to those in metazoa. They could not conceive of motion without articulation, tendons, and muscles; nor of food absorption without an alimentary tract, and they were so impressed with the ideas of what they thought they ought to see that they were convinced that they really saw many of the complicated structures possessed by metazoa. For example, the contractile vacuole, a characteristic pulsating vesicle of the protozoa, discovered by Joblot in 1754 was thought by many to be lungs, other vacuoles were said to be stomachs, the mouths were often seen and the rest of the alimentary tract was supplied from the imagination; the red pigment spots of many forms were interpreted as true eyes, etc. There were many opponents to these views, however, and the idea of the cell being the unit of structure, which was advanced by Schleiden in 1838, determine the fact that protozoa were single cells with no definite ructure.

rt I, p. 7.

of this historical sketch is abstracted from Calkins' excellent review in on "The Protozoa."

Part I. Historical Sketch.

With the publications of Dujardin (1835–41) a correct idea of the structural simplicity of the microörganisms gained ground. But for some time after, the controversy regarding the simple nature of protozoa was strenuously carried on. It is a most instructive bit of history in research work, showing how the lack of minute observation, the exercise of a too vivid imagination, and the close reasoning from analogy may lead one astray, while the proper use of these functions may bring out the truth.

Kölliker, Bütschli, Engelmann, and Hertwig, with many others (1870–80) finally demonstrated fully the unicellular nature of the protozoa.

The most important characteristic of a protozoön, its life history, was first partially made out by Trembley in 1744–47. Bütschli helped determine the sexual activities of the members of this group, while Maupas (1889) was the first to demonstrate the conditions leading to their conjugation.

Origin of Protozoa.—Though Leeuwenhoeck and one or two others believed that the animalcules developed from minute eggs or germs, the great majority of investigators thought that these low forms of life arose by spontaneous generation,[1] and it was not until late in the nineteenth century that it was finally proved that under known conditions each living organism arises from a specific spore or its prototype. When and how life began no one is yet able to say. That spontaneous generation did take place in the remote past is possible, that it may even be taking place now under unknown conditions is conceivable, but all such ideas are purely hypothetical.

Though it was known comparatively early in the study of protozoa that many forms grow on and in the higher animals and plants as parasites and that probably they have an etiological relationship to certain diseases, it was not until recently that definite forms were shown to be the cause of definite diseases in human beings. Up to the present time, however, the pathogenic forms worked out are so few, that in their further study and in the study of new forms we must still find many of our analogies in the more distantly related but better known non-pathogenic types. For this reason we include in this section some types of the common protozoa which are easily obtained for class study.

Materials Required for the Study of Protozoa.—Most of the apparatus and chemicals described in Part I as necessary for the study of bacteria are also used in examining protozoa. Attention may be called to the following essential things:

Small glass pipettes with rubber caps. Some of these should be very finely drawn out for the purpose of isolating individual protozoa in fluid media.

Platinum needles.

Shallow glass dishes with ground-glass covers for studying large numbers of protozoa in fluid media.

Cover-glasses and plain hollow glass slides.

Petri dishes for the "pure mixed" cultures of amebæ.

Glass jars, with ground-glass covers, for holding fixing and staining fluids.

A microscope similar to the one described under bacteria.

Drawing materials.

The general **fixing fluids** are: (1) *Sublimate alcohol*, two parts of concentrated watery corrosive sublimate solution and one part absolute alcohol; 5 per cent. glacial acetic acid may be added to this mixture just before using. For the use of this in smear and section preparations see page 537. Saturated sublimate + 5 per cent. glacial acetic acid is also good as a fixative.

(2) Two per cent. *osmic acid* (to be kept in a red glass with a ground-glass stopper). Moist smears are exposed to its fumes for a few seconds, small

[1] See Part I, Introduction.

pieces for sections, four to eight hours, then carried through the various alcohols and xylol and mounted or embedded in the usual way.

(3) *Hermann's Fluid.*—Platinum chloride 15 cc., a 1 per cent. solution osmic acid 4 c.c., a 2 per cent. solution, glacial acetic acid 1 c.c. Moist spreads may be fixed for several minutes; very small pieces of tissue for twenty-four hours.

(4) *Zenker's Fluid.*—Add to a solution of Müller (bichromate of potash, 2–2½ parts; sulphate of soda, 1 part; water, 100 parts) 5 per cent. of saturated sublimate solution and, when ready to use, 5 per cent. of glacial acetic acid. Moist spreads are fixed for one to five minutes, small pieces of tissue for three to twelve hours. They are then washed with water or put immediately into successive alcohols, as given on p. 625.

Staining Solutions:[1]

1. Giemsa's solution (see p. 624).
2. Loeffler's flagella mordant (see p. 35).
3. Delafield's hæmatoxylin (see Lee's Vade-mecum).
4. Carbol-fuchsin (see p. 33).
5. Iron-hæmatoxylin, Heidenhain (see below).
6. Basic fuchsin—saturated alcoholic solution.
7. Methylene blue—saturated alcoholic solution.
8. Eosin, watery solution (10 per cent.).
9. Bordeaux red, weak watery solution.

Heidenhain's iron hæmatoxylin stain is as follows:

(*a*) Mordant and differentiating fluid: Iron oxydammonium sulphate, 2.5 g.; distilled water, 100 c.c. (*b*) Staining fluid: Hæmatoxylin, 1 g.; alcohol, 10 c.c.; distilled H_2O, 90 c.c. (To be kept in a red bottle and allowed to stand for about four weeks before using.) For use see page 537.

Other fluids used:

Physiological salt solution (0.6–0.8 per cent.).
Sodium citrate solution (2.5–5 per cent.).
Iodine alcohol (iodine added until color is a clear brown).
Acid alcohol (0.1 c.c. HCl in 100 c.c. of 70 per cent. alcohol).
Alcohols 60, 70, 95, and 100 per cent.
Xylol for clearing.
Paraffins for embedding (see p. 625).
Cedar oil, or other paraffin solvents.
Canada balsam for mounting.

GENERAL CHARACTERISTICS OF PROTOZOA.

Morphology.—Shape.—The shape of protozoa varies so widely that no general description will fit all types.

Size.—Their size, too, varies within wide limits. Indeed, some forms appear to be invisible even under the highest magnification known, while the largest varieties known are two-thirds of an inch long.

The Cytoplasm.—The cytoplasm varies greatly in composition and structure according to the stage of development and the surrounding conditions. It consists of a mixture of substances, the most important of which belong to the proteids. It is more or less fluid, but, because of differences in the density and solubility of the several parts, it often

[1] The formulas for most of these stains are given in Part I under staining methods. The solutions may be obtained ready for use from Grübler, Leipzig, or by his agents throughout the world.

presents an alveolar, linear, or granular appearance, which may come out clearly in fixed and stained specimens, but is usually not well seen in the living cells. Frequently the protozoan cytoplasm is differentiated into a concentrated, viscid, more homogeneous, or hyaline outer layer called the *ectoplasm* and a more fluid granular central portion called the *entoplasm*. These two portions have different functions. The ectoplasm helps introduce and excrete food and air, therefore it becomes modified to help form the various organs of motion, contraction, and prehension. These organs are pseudopods (false feet), flagella (whip-like threads), cilia (hair filaments), suctorial tubules (through which food passes), and myonemes (contractile part of the ectoplasm found in fusoria, gregarines, and a few flagellates). Other organs, or organelles as they are called by some, are found in certain species, such as a definite oral place for the ingestion of food (cytostom), with sometimes a curved opening leading to the entoplasm; and a special anal part where the indigestible portion is dejected (cytopyge). In rare cases definite parts sensitive to light, the so-called pigment spots (euglena) are developed. The entoplasm digests the food and contains the nucleus. It may contain various granules which have been given special names as microsomes, plasmosomes, etc. These are generally products of food metabolism. The entoplasm also contains many different-sized vacuoles which serve as food digestors, and hence contain digestive ferments. The so-called contractile vacuoles which periodically fill and empty themselves may be considered as excretory organelles.

Further, fibrils of elastic consistency may often be demonstrated in the cytoplasm. These are probably instrumental in helping motion. Other substances are seen from time to time in the entoplasm, such as bacteria, red blood cells, fatty granular pigments, bubbles of gas, crystals, etc. Some protozoa secrete solid skeletal substances in or on the ectoplasm, as the chalky shells of *Foraminifera*, and the silicious framework of *Radiolaria*, etc. But these species, as far as is known, belong to the non-pathogenic protozoa.

The Nucleus.—The second element of a protozoön that is always present is the nucleus (or the nuclear substance), which varies in size, number, and structure according to the species and the stage of development. The simplest morphologic nucleus is a vesicular body which is differentiated from the cytoplasm by its essential constituent chromatin, so called because it has a strong affinity for certain basic staining materials. Chromatin consists mostly of nuclein and appears in the form of smaller or larger granules, masses, or rods. Though always having the same general staining characteristics, chromatin is composed of many substances having different physiologic as well as chemic activities.

Generally, the chromatin particles are mixed with a second less intensely staining substance with more of an affinity for acid stains, called plastin or paranuclein, similar to the substance from which the true nucleolus of the metazoan cell seems to be formed. This

substance may appear in one or more distinct rounded bodies. Most of the chromatic substances of the nucleus in many protozoa are often massed together in an intensely-staining ball-like body called the karyosome which undergoes various cyclic changes during the growth and development of the organism. The centrosome is generally embedded in the karyosome; the latter, indeed, is often simply the centrosome and attraction sphere. The chromatin and plastin lie embedded in a third substance in the form of an achromatic network called linin which is closely related to the cytoplasmic network. This network is filled with the so-called nuclear sap. There may or may not be a definite nuclear membrane. Sometimes there is no definitely structured nucleus, but the nuclear substance in the form of small chromatin masses or granules is distributed throughout the cytoplasm (the so-called "distributed nucleus")

Somatic and Generative Chromatin.—It has been shown that some chromatin substances of the cell have physiologic properties different from others. At times substances which have only vegetative properties are active, forming the so-called somatic or trophic chromatin; at other times, substances appear during sexual activities called generative or sexual or idio-chromatin, and from these the vegetative (somatic) chromatin for the new cells is again formed. In the ciliata both these chromatin elements are present as distinct morphologic bodies during the entire life of the organism, the somatic form in the macronucleus and the generative form in the micro-nucleus.

Chromidia (Fig. 182c, p. 584.—The chromatin elements, in the form of granules, small irregular masses, threads, network, etc., which pass from the nucleus into the cytoplasm, or which at times are, possibly, formed in the cytoplasm, were named "Chromidien" by R. Hertwig, who in 1899 first described their appearance. Their function in generative processes was demonstrated in 1903 by Schaudinn. During their formation the nucleus may entirely disappear, so that morphologically the cell may be considered non-nuclear. At a certain time thereafter new typical nuclei may be formed from these chromidial substances.

Locomotor Nucleus (*Kinetic Nucleus*).—In flagellates still another definite physiologic chromatin is seen in the small body called the kinetic nucleus (Fig. 175, p. 562), which is either apart from or merged into a smaller body, the blepharoplast forming the root of the flagellum. The kinetic nucleus is so called because it produces the locomotor apparatus. Both the kinetic and trophic nuclei may contain somatic and generative chromatin at the same time.

The Centrosome.—This is a small body which is always present in metazoan cells, playing an important part in cell division, but it has not been demonstrated as a morphologic entity in many varieties of protozoa; part of the karyosome, however, may take its place, or there may always be a true centrosome within the karyosome. Whenever a centrosome appears in protozoa, it has its origin in the nucleus, resembling in this the kinetic nucleus and blepharoplast. All these

four bodies, therefore, centrosome, blepharoplast, kinetic nucleus, and karyosome, may be considered as having a similar morphologic origin.

Vital Phenomena.—In common with all other living organisms, protozoa possess the essential functions of irritability, nutrition, respiration, and reproduction.

Irritability or the reaction to external stimuli of nerve response. All protozoa react in certain characteristic ways toward chemic, mechanic, and electric stimuli. Many are affected by light, while probably none react to sound. They manifest the reaction usually by motion of some sort. When toward the object of irritation, the reaction is said to be a positive taxis; when away from it, the reaction is called negative taxis. Most animal parasites, especially the higher forms, exert a positive taxis for leukocytes, principally for the large mononuclears and the eosinophiles. This fact is made use of in clinical diagnosis. Objects suitable for food cause a positive chemotaxis.[1]

Nutrition in protozoa, as in the higher animals, consists in the ingestion and digestion of food and the ejection or excretion of waste; that is, in constructive and destructive activities. Many protozoa, especially the pathogenic forms, absorb fluid food directly through the body wall; but the majority take in solid food, such as small animal or vegetable organisms and organic waste, some through more or less definite regions of the body, others through any part of the surface by extending pseudopodia and entirely surrounding the food object, forming a so-called gastric vacuole.

After the food is digested the waste products are excreted. Where no known excretory organ exists (as in the sporozoa and some other forms), the removal of the waste probably takes place by osmosis through the wall, in the same way that fluid food is taken in. In most protozoa, however, there are special structures called the contractile vacuoles which regularly eject fluid substances to the outside of the organism. In life, this vacuole is a clear spherical area in the entoplasm. As it becomes filled with fluid it grows to a certain size and then suddenly bursts. Vacuoles are generally variable in position and number. In some forms they move about with the entoplasm, in others they remain stationary. In these latter there is generally a more or less definite system of canals leading to the contractile vacuole which empties its contents into a reservoir, and from this the waste passes by a definite opening to the outside of the body.

Respiration.—It is supposed that the contractile vacuole has a respiratory as well as an excretory function. The interchange of gases is always going on, if not through a contractile vacuole, then by osmosis through any part of the wall.

Growth and Reproduction.—When the new protoplasm elaborated by the digestion of food exceeds the waste products formed, growth results. In this process the nucleus plays the most essential part. Under favorable conditions, new protoplasm is constructed rapidly,

[1] See Jennings on Behavior in Lower Organisms. New York: Macmillan & Co., 1906.

and the mass increases faster than the surface. This changed relation between internal protoplasm and its surface, according to Spencer, initiates cell division. The changes generally appear first in the nucleus. The simplest variety of reproduction is a two-celled fission which may be either longitudinal or transverse, either of which may be direct (amitotic) or indirect (mitotic). A modification of equal fission is the so-called budding division when a smaller piece breaks off from a larger. This budding occurs on the surface of the organisms and may be single or multiple. When growth occurs so that fission is for a time incomplete, one cytoplasm containing several nuclei which finally separate into as many daughter organisms, the process is called multiplicative reproduction, or brood formation. It has also been called internal budding. In the most extreme cases of multiplicative reproduction as it occurs among sporozoa the mother cell with its nucleus separates simultaneously into large numbers of tiny daughter cells. Such a process when it occurs without conjugation and encystment is called schizogony and the new cells are called merozoites. When such a multiplicative division occurs (generally after fertilization) within a cyst, it is spoken of as sporogeny and the new cells are called sporozoites. In this process the entire substance of the body may take part or there may be a residual portion left which does not divide. This finally disappears.

Sexual Phenomena.—Sexual phenomena (Syngamy) fundamentally similar to those seen in metazoa have been observed in all groups of protozoa studied. The reproduction by the usual division or budding is interrupted at certain times in the life history of each organism and individuals come together in such a way that their nuclei fuse after having undergone characteristic reduction divisions. When the union is permanent, we speak of it as copulation and liken the process to that of the fecundation of the ovum by a spermatozoön. When the union is transient we call it conjugation. Here the two cells fuse for a time when the nuclei interchange protoplasm and then the cells separate and each one continues to grow and divide independently. When in a partly divided cell or in an apparently single cell, two nuclei, after undergoing reduction division, or its like, fuse, the process is called autogamy. The developmental cycle of a protozoan consists of all the changes which occur in its growth from one act of fertilization to another (Fig. 186, p. 590). According to Calkins, such a developmental cycle as a whole should be considered the individual and should be made the basis for species rather than any part or parts of it. Many protozoa carry on the sexual part of their life cycle in one host and the asexual part in another. It is thought by some that the so-called intermediate hosts in many instances, if not all, were the original hosts of the parasites, the change possibly being due to the fact that as the parasites developed they found soil more favorable for certain stages in their growth in new hosts.

Cyst Formation.—The function of encystment is a marked characteristic of all protozoa. It is the means developed by these organ-

isms for surviving unsuitable environments. If they do not get the required amount of water or air or suitable food they cease their special movements, round out into more or less of a sphere and form a resisting membrane of chitin within which they may live for a long time, withstanding periods of desiccation, extreme heat and cold, and they may be blown about as dust until they find conditions again favorable for renewed growth when water is absorbed, the cyst is ruptured and active life begins anew. In parasitic forms encystment plays an important part in the passage from the old host to the new. The majority of forms would not be able to exist outside of the body of the host without having some protective membrane. The cyst may be formed simply for protection from drought, etc., when it is called a hypnocyst, from which the organism may emerge in about the same form as when it encysted; or the cyst may precede reproduction by spore formation or simple division, when it is called a sporocyst. In either case it may consist of a simple wall or it may be formed of several walls to enable it to resist prolonged desiccation, when it is called a resting cyst.

Natural Habitat.—On account of this power to form lasting cysts, protozoa have a world-wide distribution. They are found in largest numbers where the climate is warm and moist, but even in Alpine and Arctic regions a few species which are able to resist long periods of drought and cold grow freely during the warm season. They are abundant in both salt and fresh waters. Finally, they are found as parasites on or in animals and plants.

Cultivation.—Protozoa are cultivated *en masse* in the large aquaria of the zoölogical laboratories, where they are mixed with the bacteria and the plants and animals usually found in the material taken to stock such aquaria.

Pure cultures such as are known among the bacteria have not been obtained with protozoa until recently, when Novy succeeded in growing certain blood flagellates in the condensation fluid of a mixture of blood and nutrient agar. Before that it was shown by Frosch and others that so-called "pure mixed" cultures of certain protozoa, especially of certain species of amebæ, could be obtained by separating them from other protozoa and feeding them on one or two varieties of known bacteria.

Though this field is an important one, comparatively little work has been done in it. Up to the present time zoölogists have studied these organisms as nearly as possible in their natural environment. They have thought that anything which disturbs the usual surroundings might lead to degeneration, or at least to involution, and hence that wrong interpretations might be drawn from phenomena observed under these circumstances.

The special methods so far used in cultivating protozoa will be considered under the descriptions of the individual organisms.

Effects of Physic and Chemic Agents.—Some of these have been already mentioned under irritability. For the physician it is espe-

cially important to know the effects of (1) temperature, (2) electricity, (3) light, (4) moisture, and the various chemicals used as (5) disinfectants.

1. Each species of protozoa has an optimum temperature at which its movements are more rapid and its growth more vigorous than at other temperatures. With increasing or decreasing temperatures, movements and growth gradually cease. In intense cold the organisms may continue to live quiescent for a long time, while with a comparatively moderate amount of heat most of them will die.

2. When a current of electricity is passed through a liquid medium most active protozoa swim with their long diameters in the direction of the lines of force to assemble behind the cathode. Most flagellates and a few ciliates, however, move toward the anode. The direction of motion has been shown by Dale to vary with the nature and concentration of the medium. This whole question has been little studied.

Slight induction shocks arrest movement, stronger ones cause contraction, stronger still will kill the protozoa.

3. Most protozoa are greatly influenced by light, some moving toward the point of greatest luminosity, others away from it. The light-seeking protozoa have green or yellow chromatophores and usually, at the anterior end, a red pigment spot. Here, as with other stimuli, there is different optimum light for different species. The violet and blue rays are more active than other parts of the spectrum in determining motion. The effect of x-rays and of radium emanations have been little studied. Most of the colorless protozoa are negative to light.

4. When protozoa are encysted while drying they will withstand long periods of desiccation. Most forms when dried quickly, remain viable much longer than when dried slowly. A certain amount of moisture, as we have said, is absolutely essential to renewed activity.

5. The effects of the usual chemic disinfectants have been very little tried on protozoa. In general what is true for bacteria in this particular is probably true for protozoa.

Chemical Composition.—The chemical composition of the bodies of animal parasites is an almost unexplored field. The ectoplasm and the cyst sacs in general are made up principally of a substance called chitin. Glycogen has been isolated from many forms. Proteolytic enzymes and acid secretion in digestive vacuoles have been demonstrated. Microchemic reactions have been studied in the individual organism.

Pathogenesis.—The pathogenic protozoa, indeed the parasitic forms, are few in numbers compared with the total number of protozoa. They exert their harmful action mainly mechanically or by the direct destruction of the special host tissue which they find suitable for food. That they may produce specific toxic substances has been demonstrated in only one or two instances, the most marked of which is that of the poison obtained in the aqueous or glycerin

extracts and the dried powder from mutton sarcosporidia which will be spoken of later.

Though in general no specific toxins have been shown to exist in pathogenic forms or to be excreted by them, the fact that there is spontaneous recovery from various protozoan infections and that a reinfection does not take place soon after, indicates that some specific toxins or substances are formed which help to produce immunity. Rössle has stated that he has obtained immune sera against infusoria; and antibodies have been demonstrated in animals which have received non-lethal doses of trypanosomes and of amœbæ.

Infection through protozoa is often accomplished by means of some of the lower animals either acting as intermediary hosts or as direct carriers of the virus.

Classification.—Broadly, protozoa are classified from two principal standpoints, the physiologic and the morphologic.

Physiologically, they are grouped according to their manner of living into saprophytic and parasitic forms.

The parasitic protozoa may be further divided into commensal and pathogenic forms. For our study the former are almost equal in importance to the latter forms because of their close relationship to the pathogenic forms and because of the possibility of their becoming pathogenic.

The classification of the protozoa in the strict sense is morphologic and is based upon variations in the motile organs. It is still in a transitional stage and it will continue to be so until the relations of the different groups are better known and until the life histories of the different species have been more minutely worked out. Hartmann has just added to the flagellata a new order made up of species taken both from the flagellates and from the sporozoa. Calkins has also announced some fundamental rearrangements; so, whatever system of classification we adopt, we may be sure that the near future will show us some changes in it. The following grouping is taken, with a few slight alterations, from the excellent article on *Protozoa* written by Calkins in Osler's Modern Medicine.

CLASSIFICATION.

PHYLUM. PROTOZOA.—Unicellular animal organisms which reproduce by division or spore-formation; solitary or united in colonies; free-living or parasitic.

SUB-PHYLUM I. SARCODINA.—Protozoa with changeable protoplasmic processes or pseudopodia.

Class I. *Rhizopoda.*—Sarcodina with pseudopodia in the form of lobose or reticulose processes, with or without shells.

Sub-class. *Amœbida.*—Pseudopodia lobose.

Order 1. Gymnamœbida.—Naked amœboid forms with lobose pseudopodia. Here are placed a few parasitic forms belonging to the genera *Amœba* and *Entamœba.*

Order 2. Thecamœbida.—Shell-bearing amœboid forms with lobose pseudopodia. One parasitic form, genus *Allogromia,* is placed in this order.

Sub-class. *Foraminifera.*—Divided into 10 orders; the various genera are salt water forms for the most part and are rarely parasitic.

Sub-class. *Mycetozoa* would be placed here were we to consider these forms, as protozoa instead of fungi. Here are placed parasitic forms, such as *Plasmodiophora, Tetramyxa, Labyrinthula,* and *Nucleophaga.*

Class II. *Heliozoa.*—The genera are confined mainly to fresh water and are never parasitic. They are subdivided into four orders according to the nature of the skeleton.

Class III. *Radiolaria.*—Salt-water forms of protozoa, never parasitic.

SUB-PHYLUM II. MASTIGOPHORA.—Protozoa with flagella.

Class I. *Flagellata.*—Small forms with from one to several flagella; with a strong tendency to form colonies.

Order 1. Monadida.—Minute forms with from one to three flagella. There is no definite mouth-opening and nutrition is holozoic, saprophytic, or parasitic. The parasites and commensals which belong to this order are species belonging to the genera *Cercomonas, Herpetomonas,* and *Trypanosoma* (appendix *Spirocheta*).

Order 2. Choanoflagellida.—With collar-like processes surrounding the base of the flagellum; not parasitic.

Order 3. Heterommastigida.—With two or more flagella of dissimilar length; the genus *Bodo* is parasitic.

Order 4. Polymastigida.—The flagella are numerous and of similar or dissimilar size. Here are several ecto- and endoparasitic forms belonging to the genera: *Costia, Tetramitus, Trichomonas, Monocercomonas, Hexamitus, Lamblia, Polymastix, Lophomonas, Trichonympha, Pyrsonympha,* and *Jœnia.*

Order 5. Euglenida.—Occasional parasites as Copromonas in frogs.

Order 6. Phytoflagellida.—Flagellates with coloring matter in the form of green, yellow, or brown, chromatophores. Frequently colonial. Here belong the most frequent sources of odors in drinking waters, the following genera being especially noteworthy: *Dinobyron, Synura,* and *Uroglena,* all colonial forms, with yellow chromatophores.

Order 7. Silicoflagellida.—A single genus of salt water mastigophora with latticed skeleton. *Distephanus,* parasitic on radiolaria.

Class II. *Dinoflagellata.*—Never parasitic.

Class III. *Cystoflagellata.*—Two genera of characteristic form. One, *Noctiluca,* is remarkable for the vivid phosphorescence which it causes.

SUB-PHYLUM III. INFUSORIA.—Protozoa with cilia.

Class I. *Ciliata.*—Cilia present at all times.

Order 1. Holotrichida.—The cilia are distributed over the surface, and there is no specialized oral apparatus known as the "adoral zone" consisting of cilia fused into "membranelles." Here are found some parasites belonging to the genera *Ichthiophthirius, Bütschlia, Anophrys, Isothrica, Dasytricha, Opalina.*

Order 2. Heterotrichida.—With cilia distributed over the general surface, and, in addition, a specialized zone in the mouth-region. Here are several well-known parasitic forms belonging to the genera *Nyctotherus, Balantidium, Entodinium, Ophryoscolex* and *Cycloposthium.*

Order 3. Hypotrichida.—The cilia are limited to the ventral surface, and are frequently fused into specialized organs of motion and touch, the cirri. There are no strictly parasitic forms.

Order 4. Peritrichida.—The cilia are greatly reduced, in some cases to the adoral zone, but additional rings may be present. Several ectoparasites belong here, especially the genera *Spirochona*, *Kentrochona*, *Lichnophora*, *Cyclochæta* and *Trichodina*.

Class II. *Suctoria.*—Infusoria with suctorial tentacles in the place of cilia except in the young phases. They are frequently ectoparasites and the young of some genera, *e. g.*, *Sphærophyra*, are internal parasites in other infusoria.

Sub-phylum IV. Sporozoa.—Protozoa without motile organs; reproduction by sporulation; always parasites.

Class I. *Telosporidia.*—Sporozoa in which the act of reproduction ends the individual's life, the entire protoplasm being used in forming spores.

Order 1. Gregarinida.—The young stages alone are cell parasites, the adult organisms living in fluids within the cavities of animal hosts. There are no human parasites.

Order 2. Coccidia.—Intracellular parasites, mainly in the epithelial cells of vertebrate and invertebrate hosts. Human parasites have been traced mainly to the genus *Coccidium*.

Order 3. Hæmosporidia.—Sporozoa of small size living in the blood corpuscles of vertebrates. Human parasites belong to the genera *Plasmodium* and *Babesia*.

Class II. *Neosporidia.*—Sporozoa in which the entire cell is not used at one time in forming spores, the latter developing while ordinary vegetative processes are carried on.

Order 4. Myxosporidia.—Neosporidia with spores containing polar capsules and anchoring threads. Here belong several genera of note, in that serious epidemics of lower animals are caused by them, *e. g.*, *Nosema*—causing pébrine diseases in silkworm, *Myxobolus*, *Myxidium*, etc.

Order 5. Sarcosporidia.—Neosporidia in which the initial stages are passed in muscle cells of vertebrates. Cysts are formed with double membranes in which kidney-shaped reproductive elements are produced. The one genus occasionally parasitic in man is *Sarcocystis*.

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CHAPTER XL.

GYMNAMŒBIDA. MYCETOZOA.

GYMNAMŒBIDA.

Introduction.—Under gymnamœbida (syn., amebæ) we include forms composed of naked, simply constructed protoplasm having the power of producing lobose pseudopodia which are used as organs of motion and of nutrition.

The pseudopodia are protoplasmic processes which are projected in irregular succession from different parts of the surface of the cell, producing in this way an irregular motion. The form of the pseudopodia varies considerably in the different species. For instance, there are broad, blunt processes or narrow, less blunted ones, and each may be short or long, single or slightly branched. The entoplasm may or may not take a share in their formation. The forms of course vary within limits according to the condition of the medium in which the amebæ are living. Movements are always called forth by some physic or chemic excitant. When such an excitant is desirable for food the pseudopods flow around it, and it is subsequently absorbed in the cytoplasm of the organism.

The members of this group may possess one nucleus or several. *Amœba binucleata* has two nuclei in the young adult stage, and *Pelomyxa palustris*, living in the bottom ooze of ponds, has an enormous number of nuclei. A marked feature of the nuclear apparatus is the formation of chromidia which, as has already been noted, may play such an important part in sexual reproduction. Generally each ameba has one contractile vacuole, but occasionally some are seen with several or with none.

Saprophytic forms belonging to this order are common. They may be found wherever there are moisture and decaying vegetable matter. The pathogenic forms are not so frequent. Because of the possibility of the still unknown causes of certain diseases (see rabies and smallpox) being organisms related to this order, it is especially important to study both saprophytic and pathogenic varieties, since a knowledge of the former which are more easily studied may help us understand obscure points in the life history of the latter.

Notwithstanding the common occurrence of saprophytic forms, the full life history of few of them has been worked out, and until the full cycle of development of any so-called ameba is known it is impossible to say whether that particular form belongs among rhizopoda or whether it is a developmental form of another group, as ameboid forms may occur at some time in the life history of all groups. It is quite

possible that some of the organisms described as belonging to this order are really members of entirely different orders. For instance, it is known that the flagellate *Trichomonas* loses its flagella before copulation and crawls about by means of short blunt pseudopods as a typical ameba.

Gymnamœbida reproduce by simple fission, by budding, and by brood formation. In the last case the reproduction is usually preceded by encystment. Schaudinn has worked out in several rhizopoda a complex life cycle, part of which is sexual and part non-sexual in character. Calkins has worked out a complete life history of *Amœba proteus*, in which secondary nuclei form within the primary ones and conjugate after leaving the latter.

Gymnamœbæ in Human Beings.—Several authors have reported the finding of amebæ in man, especially in so-called tropical, ulcerative, or amebic dysentery, but as the first descriptions were incomplete and the laws of nomenclature were not strictly followed there resulted many synonyms for the same species and many species bearing the same name.

Only four out of all those mentioned have been described with enough minuteness to be considered as distinct species. These are *Entameba histolytica*, the form described by Schaudinn from tropical dysentery and considered by him the cause of that disease; (2) *Entameba coli*, the kind found in normal human intestines by Schaudinn and thought by him to be harmless; (3) *Entameba buccalis*, found by Prowazek in tartar of teeth and considered harmless; (4) *Entameba tetragena*, a form found by Viereck in certain cases of tropical dysentery.

The chief differential characteristics of these amebæ, as reported by various investigators, are given in the table on p. 534.

Historical Note.—Stiles has given a detailed history of the generic name *Amœba* and of the specific one *Amœba proteus*, and, finally, of the naming of the intestinal amebæ. He shows why the name *Entamœba* should be given to the genus described by Lamdl and Lösch.

This article illustrates very forcibly the absurdity of bringing forth new names for organisms only half studied and of claiming that such organisms belong to new genera.

The first report on intestinal amebæ of man was made by Lamdl in 1860 who announced the presence of ameboid forms in the intestinal mucus of a child who had died from enteritis. Supposedly the same forms were more fully described by Lösch in 1875 under the name *Amœba coli;* Lösch found his organisms in stools of a patient suffering from chronic dysentery and he succeeded by rectal injections in producing superficial ulceration in the large intestines of dogs. He therefore claimed that this organism is the cause of dysentery. His work was corroborated by many observers. In the meantime, amebæ were found in diseases other than dysentery, and Grassi in 1879 reported them in the healthy intestines. The work of Kartulis, however (1886), helped largely to establish the fact that amebæ play an important part in the etiology of dysentery in Egypt. He was the first to find the organism in abscess of the liver in tropical dysentery. In our own country among the most important workers in this field are Councilman and Lafleur (1891). They conclude that amebic dysentery should be regarded etiologically, clinically, and anatomically as a distinct disease. They disap-

TABLE GIVING CHIEF DIFFERENCES CITED BETWEEN MEMBERS OF THE GENUS ENTAMŒBA PARASITIC IN MAN.

Name of species and date described	Size of ameboid forms at rest	Pseudopods and motility	Cytoplasm	Nucleus	Cysts	Propagation	Cultures	Effect on host	Other points
Ent. buccalis, Prowazek. 1904.	6–32μ	1 to several, not described. Motion moderate.	Distinct ento- and ectoplasm. Former filled with food vacuoles, and some greenish granules. No contractile vacuole.	Small amount of chromatin in center and about periph. In life shows thick glistening memb.	Contain many nuclei (not further described).	1. Simple amitotic (?) division. 2. Formation of chromidia and many daughter nuclei after encysting.	Not made.	None so far as known, tho' forms described in abscess of jaw may be pathogenic varieties of this species.	
Ent. coli, Losch. Described as species distinct from pathogenic form by Schaudinn in 1903.	8–50μ majority 10–20μ	Rounded and small. Motion sluggish.	Ectoplasm not distinct unless in motion, then thin, pale gray and only slightly refractive. Entoplasm finely granular with few vacuoles which are never contractile. Red blood cells, none or few.	Distinct because of large karyosome, large amount of distributed chromatin and definite membrane.	Contain eight nuclei which show distinctly through the more or less irregularly thickened wall.	1. Simple binary fission (mitotic). 2. Autogamous sexual reproduction within cyst.	Claimed by some, denied by others.	Non-pathogenic.	Craig differentiates ameboid form by Oliver's modification of Wright's stain: ectoplasm is light blue, entoplasm dark blue, nucleus crimson.
Ent. histolytica, Schaudinn. 1903.	20–70μ majority 25–35μ	Long finger-shaped forms highly refractive. Motion lively.	Ecto- and entoplasma distinct. Former homogeneous. Latter granular, and generally contain many vacuoles and red blood cells	Faint because of small karyosome, small amount of distributed chromatin and delicate membrane.	Tiny with thick wall through which stains do not penetrate, hence structure not known	1. Simple binary fission (mitotic). 2. Chromidia formation with cysts produced by budding.	Claimed by some, denied by others.	Pathogenic for man, dogs, and cats.	With above stain, ectoplasm dark blue or violet, entoplasm light blue, nucleus delicate pink or red.
Ent. tetragena. Viereck. 1907.	Same as Ent. histolytica.	Similar to Ent. histolytica.	Similar to histolytica.	Distinct karyosome surrounded by clear space and this ring of chromatin	Four nuclei Cyst wall similar to that of Ent. coli.	1. Primitive mitosis. 2. Autogamous sexual reproduction within cyst	Negative results reported	Pathogenic for man and cats.	

prove, however, of the name *Amœba coli* and propose the name *Amœba dysenteriæ* for the pathogenic form; but as they do not show in any way than by its pathogenesis that the species they describe is a new one, their name, according to the rules of zoölogical nomenclature, cannot be accepted. Harris's work, too, is important in showing an etiologic relationship between amebæ and a certain form of dysentery, but neither did he describe the morphology of his organism minutely enough to identify it with Schaudinn's histolytica which is described below. Casagrandi and Barbagallo in 1897 were the first to claim that the amebæ so far described in man show differences enough from the fresh-water amebæ to belong to a new genus. They therefore created the genus *Entamœba* and gave the specific name *Entamœba hominis* to amebæ of the *Amœba coli* type. Schaudinn and Stiles agree with them as to the generic name, but consider that the correct specific name is complicated by the fact that there are different species in this group. Many observers (Kartulis, Councilman and Lafleur, Quincke and Roos, Kruse and Pasquale), have considered that there are different varieties in the human intestines, but they have given no morphologic differences distinct enough to classify such varieties. Schaudinn is the only one who seems to have shown quite clearly (1903) that at least one species among them is pathogenic and one non-pathogenic. The latter, which he found in normal human intestines, he considers resembles those already described as *Amœba coli;* therefore he gives it the name *Entomœba coli;* while the former, which he found exclusively in ulcerative tropical dysentery, he calls *Entamœba histolytica.*

The different views upon the relationship to disease of amebæ found in the human intestines may be summarized as follows:

1. That the amebæ in man have no pathogenic properties, hence are not the cause of amebic dysentery. (Cunningham, Grassi, Celli and Fiocca, Casagrandi and Barbagallo, and others.)
2. That any intestinal ameba may become pathogenic and cause the specific malady known as amebic dysentery. (Musgrave, Clegg, and others.)
3. That amebæ are able to keep up a pre-existing inflammation. This was the original view advanced by Lösch when he described the most commonly cited form, *Amœba coli,* and several authors have followed Lösch in this opinion.
4. That more than one species of amebæ are found in man, at least one pathogenic, and one non-pathogenic. (Kartulis, Councilman and Lafleur, Quincke and Roos, Strong, Schaudinn, Craig, and others.)

The study of bacillary dysentery by Shiga, Kruse, Flexner, and others (see under bacillary dysentery) has demonstrated that there are at least two forms of dysentery, one produced by amebæ and the other by bacilli, and from the work on the former it now seems certain that it is produced by a specific form of amebæ.

Because of the incomplete earlier descriptions, however, we cannot yet decide in what percentage of cases Schaudinn's non-virulent form is found and in what percentage his virulent. The cases described by Councilman and Lafleur and by Harris in our country were probably all due to the *histolytica,* especially as they mention the distinct ecto- and entoplasm of their organisms; but as they do not go into detail of its life history we cannot be absolutely sure. Schaudinn speaks of finding the *coli* accompanying the *histolytica* in cases of true amebic dysentery and of finding them increased in numbers in cases of simple diarrhœa, but does not mention their presence in liver abscesses. The whole work of Schaudinn needs more corroboration, but until that time, his classification and descriptions must be provisionally accepted. Lesage, Craig, and Wenyon claim to have corroborated more or less of Schaudinn's work.

Amebæ have been reported in teeth cement and in carious teeth as well as in abscesses of the jaw. Flexner in 1892 described an amebic organism in the latter condition, and considered it identical with the organism described by Lösch, Councilman and Lafleur as *Amœba coli.* In the same year Kartulis

described similar organisms found in similar lesions. Gross and Sternberg found them in tartar of teeth. Prowazek (1904), however, is the first to have separated a mouth ameba as a distinct morphologic species under the name *Entamœba buccalis.*

Comparatively recently, successful cultures have been made of amebæ obtained from the intestines of man and other animals, as well as from certain fruits and vegetables.

Musgrave and Clegg (1904) studied amebæ in the Philippines by the culture method and came to the conclusion that forms obtained from various sources were probably all a single species.

Kartulis (1885) reported growing pure cultures of the amebæ from a bacteria-free liver abscess in dysentery. He used straw decoction as a medium. In 1895 Celli and Fiocca claimed to have obtained a pure growth of amebæ from an abscess of the liver, free from bacteria, upon an alkaline medium containing *Fucus crispus.* But the organism did not reproduce in transplants. In 1898 Tsujitani reported the pure development of encysted cultures of amebæ. He took old cultures of a favorable symbiotic organism, and heated them for an hour at 60° C. (to kill organism). These dead organisms were then inoculated with encysted amebæ, and development occurred, though not so luxuriantly as with a living organism.

Walker made an extensive cultural study of 40 strains of amebæ obtained from human dysentery and from other sources and agreed with Musgrave and Clegg in considering the pathogenic and the non-pathogenic human forms as single species.

The results from cultural work alone, however, cannot be accepted as disproving Schaudinn's work. The organisms must be studied comparatively in their human habitat and in cultures before judgment can be passed upon his work.

Sites of Amebæ in the Human Body.—Intestines and neighboring tissues; abdominal cavity; abscess of liver, lung, pleura, and mouth; necrosis of jaw-bone; urine; tartar of teeth.

Material and Methods for Study.—Fresh material containing the pathogenic amebæ is so seldom on hand in the northern part of this country that it cannot be counted on. The *Entamœba buccalis* may be found sometimes in the tartar collections about human teeth, but the demonstration is often unsatisfactory. If found it must be examined immediately on a warm stage in order to detect motion. The non-pathogenic form in human intestines might be obtained after administration of a saline cathartic, but generally one must depend upon saprophytic forms for work with students, or upon cultures obtained from cases of amœbic dysentery. Material rich in saprophytic forms may be obtained from an infusion in water of lettuce, cabbage, potato skins, or other vegetable material. Such an infusion should be made a week or two before it is needed, when it will be found that the pellicle which forms contains many varieties of protozoa and bacteria, among which are generally large numbers of ameboid forms. Often one may get good material from the fæces of many of the lower animals, such as the lizard, toad, or guinea-pig.

If one has material containing human intestinal amebæ, cats may be fed with the cysts in order to obtain a new supply. The amebæ should be examined in both the fresh and fixed condition. Cultures may also be made as described below.

Examination of the Fresh Material.—The study of the living amebæ is extremely important. This may be done by making a hanging drop or hanging mass (p. 41) from fluid containing amebæ. The size, kind of motion, frequency of pulsation of contractile vacuole, and as much of the cell contents as possible should be noted.

The stools should be examined on the warm stage as soon as possible after their passage (not later than two hours), and should be kept at blood heat until examined. A platinum loopful of material should be taken from the slimy masses in the thinner part of the fæces, diluted with physiologic salt solution, covered with a cover-glass, and examined under moderate magnification.

Harris found that a drop of a watery solution of toluidine blue added to a small particle of the fæces stains the entoplasm of the amebæ at once and the ectoplasm a few minutes later. The amebæ seem to be quickly killed and often when natural forms are beautifully preserved the coverslips, after being washed in water and mounted in Farrant's medium, may be preserved for months, but after a time the stain completely fades.

Permanent Preparations.—Thin films are made on glass slides or cover-glasses, and immediately, before they are allowed to dry, they are placed in the fixing solution. Cover-glasses may float, film down, on the surface of the fixative. Among the best fixatives are the following:

1. Hot sublimate alcohol (50° C.) (Schaudinn), or saturate sublimate, to which 5 per cent. glacial acetic acid may be added. The preparation should remain in it a few seconds, then should be washed for one-half hour in 60 per cent. iodine-alcohol, and then placed in 70 per cent. alcohol. They may remain here for an indefinite time, until they are to be stained, when they are rinsed in distilled water and then placed in the staining fluid.
2. Zenker's fluid (p. 522). See p. 625, for use.
3. Hot (50° C.) Hermann's fluid (see p. 522) for a few seconds, washed in distilled water for ten minutes, in 60 per cent. alcohol, and then in 70 per cent. alcohol, from which they may be stained at any time.
4. Methyl alcohol for a few seconds.

Stains.—Among the many good staining methods the following may be mentioned:

1. Thin Delafield's hæmatoxylin from one-half to several hours, then washed in water. (If over-stained, the preparation may be differentiated in acid alcohol, controlling under the microscope, then washed in water.) The film or section is then passed successively through 70 to 95 per cent. and 100 per cent. alcohol, absolute alcohol+xylol, xylol, cedar oil, or Canada balsam.
2. Heidenhain's iron hæmatoxylin (see p. 522). The smear is put from distilled water into the iron-alum mordant for 4 to 12 hours, or overnight; well washed in distilled water; in stain from 2 to 24 hours, excess washed out in the iron mordant, controlled under the microscope (as decolorization occurs very quickly), until the nucleus is sharply differentiated; the chromatin of the nucleus must be a deep blue-black, and the cytoplasm a light gray; then a thorough washing in tap water and passage through the alcohols and xylol, and in Canada balsam, or cedar oil for mounting.
3. After fixation in methyl alcohol one may use Giemsa's staining method (see p. 624), or a modification of the method suggested by Van Gieson for staining the Negri bodies in smears (see p. 624).
4. Mallory's eosin methylene blue method (see p. 625).

Masses containing amebæ, as mucous flakes or portions of the intestinal or liver abscess wall in amebic dysentery, or pieces of decaying vegetable may be fixed *in toto* in hot sublimate alcohol for one-half hour, washed in iodine-alcohol for 24 hours, passed through the different strength alcohols and embedded in paraffin (see p. 625) for section-cutting if desired.

Mallory and Wright recommend the following method for tissues:

1. Harden in alcohol. 2. Stain sections in a saturated aqueous solution of thionin three to five minutes. 3. Differentiate in a 2 per cent. aqueous solution of oxalic acid for one-half to one minute. 4. Wash in water. 5. Dehydrate in alcohol. 6. Clear in oleum origani cretici. 7. Wash off with xylol. 8. Xylol balsam.

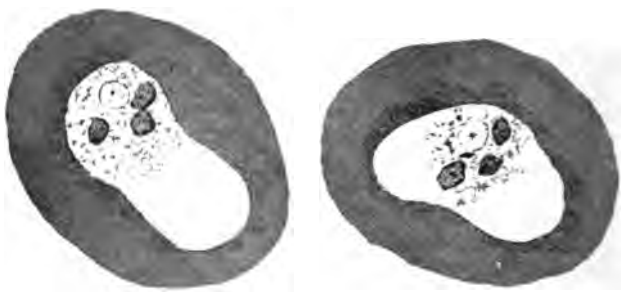
Mallory's eosin methylene-blue method is also very good for sections.

Cultures of Amebæ may be made in the following way: From the material containing amebæ a small loopful is removed with a platinum wire and isolated spots are touched over the surface of the following media poured in sterile Petri dishes: Agar 1.0, tap water 90.0, ordinary nutrient broth 10.0, mixed and sterilized in the regular way. It should be slightly alkaline to phenolphthalein (1 per cent.). If necessary, fæces contents may be thinned with physiologic salt solution before planting. In one to several days at 25° C. the amebæ with the accompanying bacteria may overgrow the entire plate. We have found that amebæ will grow as well upon nutrient agar—better with certain bacteria—as on the special media just mentioned. Klatsch preparations may be made of these cultures, or small pieces of agar and culture may be embedded entire. From such a culture the "pure mixed" cultures of Frosch may be made as follows: The amebæ which have crept out to the periphery of the growth are taken out with their accompanying bacteria and transplanted. Usually one or two organisms favorable for the growth of the amebæ accompany them and in this way one may finally get the amebæ growing with one definite bacterium. We have isolated from a culture a single ameba unaccompanied by bacteria by the following simple method: Under the low-power lens with a fine platinum loop an isolated ameba is drawn to the edge of the agar plate. When it is well separated a disc of agar containing it is cut out following the margin of the objective and is transferred to a fresh agar plate. A very small quantity of a desired bacterum is now added to the disk near the ameba, and a "pure mixed" culture results.

Comparative Characteristics of Amebæ from Human Sources.—The chief properties of these organisms so far described may be learned by studying the table on p. 534.

Morphology.—The morphologic characteristics of the ameboid stage, as described by various observers, seem not to have been minutely enough studied to be depended upon in differentiating the species.

FIG. 166



Entamœba histolytica (Schaudinn) from the stool of a dysentery patient. The same individua showing two successive movements. The entoplasm contains the nucleus and three red blood cells. Enlarged 500 : 1. After Jürgens (from Kisskalt and Hartmann).

Moreover, descriptions have differed markedly. While Schaudinn and others, especially Craig of the more recent writers, say that it is easy to differentiate between the ameboid forms of histolytica and coli, Musgrave and Clegg, Strong, and others say the points of difference are not marked; but there has not been enough work done to disprove Schaudinn's work; hence his observations and those of his followers

must be provisionally accepted until further study of each species under varying conditions shows whether or not these characteristics hold.

The observations of Schaudinn and others may be summarized as follows: (1) *Ent. coli* is, on the whole, smaller than *Ent. histolytica;* (2) its ectoplasm is so small in amount and so slightly differentiated that it is only seen when the organism puts forth pseudopods, while the cortical zone of the *Ent. histolytica* is wider and is distinctly differentiated from the entoplasm; (3) the pseudopods of the former are small, rounded, delicate, and not highly refractive, those of the latter are larger, finger-shaped, firmer, and more highly refractive, thus indicating the power of the organism to penetrate its host's tissues; (4) the nucleus of *Ent. coli* is very distinct in life as well as in stained spreads, due to a definite membrane, a more distinct karyosome, and much chromatin which is distributed throughout the nucleus with more of a collection about the periphery; the nucleus of *Ent. histolytic*, on the other hand, is seen with difficulty during life, and stains faintly, owing to its delicate membrane, its small amount of chromatin, and small karyosome, the chromatin is collected about the karyosome and the periphery of the nucleus, the nucleus, moreover, is much more variable in shape, in the active organism than is that of the *Ent. coli;* (5) the entoplasm of *Ent. coli* is less granular and vacuolated and contains fewer red blood cells than that of *Ent. histolytica* which sometimes shows immense numbers of these blood cells.

The above points of difference cited for organisms in the ameboid stage may hold for forms living in the human intestines; but we have found that organisms from widely different sources (*e. g.*, intestines of guinea-pigs and of dogs from New York and of humans from the Philippines) when grown with a favorable bacterium in the thermostat at body temperature may show appearances similar to each other and similar also to those described by Schaudinn for *Ent. histolytica.* As already said, therefore, more corroborative work seems to be needed before we accept the above observations as being the whole truth.

Reproduction.—The most important point of difference between these organisms, according to Schaudinn, and the one upon which he rightly bases his classification into different species is the manner of propagation.

In the vegetative stage probably all these forms divide by a primitive mitosis, though Schaudinn, Craig, and others saw only amitosis. All of our culture forms divide by mitosis and many observers have recently reported similar division in related forms. *Ent. coli* in the vegetative stage may also divide by breaking up (schizogony), into, at the most, eight daughter cells. In the latter instance the nucleus undergoes a somewhat complicated process of division. At first it increases in size and then the chromatin gathers together about its periphery into eight particles, the nuclear membrane disappears, and the chromatin masses lie in the cytoplasm which separates into eight

parts about each nuclear mass, forming the eight daughter amebæ which creep away.

The vegetative stage of each intestinal organism takes place in the upper part of the intestines; as the fæces become thicker, most of the vegetative forms die off, while some pass on to permanent cyst formation. As with many coccidia, parasitic amebæ may pass through a long period of vegetative life before entering upon a sexual phase wherein forms are produced capable of infecting a new host. The length of this period depends upon a number of circumstances. Under conditions favorable for the growth of the ameba, as in cases of diarrhœa, the vegetative phase is considerably lengthened, while in healthy intestines, as the amebæ pass down with the thickening fæces, the infecting cysts are more or less quickly formed.

Ent. histolytica during the vegetative stage may multiply by budding as well as by binary fission, but never by multiple division, as does *Ent. coli*.

Sexual Phenomena.—The most marked difference between the two forms, according to Schaudinn, is seen in their cyst formation and accompanying sexual phenomena. Schaudinn has described the process as follows.

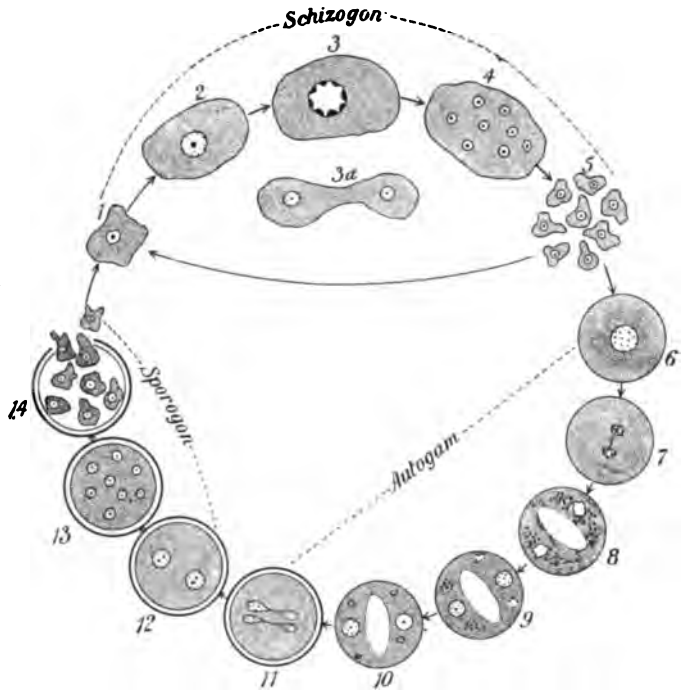
Within the cyst of *Ent. coli* the special form of conjugation known as autogamy (Fig. 167) takes place. The cell becomes rounded, rids itself of all foreign matter, and forms a mucous wall about itself. Then the nucleus divides by mitosis into two daughter nuclei, which separate one from the other and between them appears a lens-shaped hole, as if two not fully divided cells were forming. These are the gametes. The nuclei give out most of their chromatic substance as chromidia into the cystoplasm and then gradually break down and become absorbed. The chromidium is generative and from it two new nuclei are formed, the sexual nuclei. These divide mitotically, forming two reduction nuclei, which gradually disappear. By this time a firm cyst membrane is produced. The central clear space in the cytoplasm disappears and the two reduced nuclei divide by a primitive mitosis with the two spindles lying parallel, and each half of one nucleus unites with the corresponding half of the other, one remaining stationary as a female nucleus and the other moving over to it as a male nucleus—thus two fructified nuclei are formed. After this autogamous phase there follows a mitotic division until eight nuclei are formed. The cyst containing these nuclei is characteristic for this species, and is not seen, according to Schaudinn, in any other intestinal parasites.

In the beginning of the large intestine of the new host the cyst wall is dissolved, the cyst contents divides into eight young amebæ (sporogony), and the cycle begins anew.

The permanent cysts of *Ent. histolytica*, on the contrary are formed in an entirely different manner. The nucleus of the ameboid form enlarges, the chromatin increases, and most of it passes out as chromidia into the cytoplasm which is finally filled, while the nucleus degenerates.

On the surface of the cytoplasm are now formed small rounded bodies of 3 to 7μ diameter. These balls, which can be seen forming in the hanging drop, produce about themselves a double-contoured membrane which after several hours takes a clear brownish-yellow color, and becomes very refractive. At this stage no structure can be made out within the ball. The rest of the ameba finally degenerates. The stained prep-

FIG. 167



Scheme of the development cycle of *Entamœba coli* (Lösch). 1–5 Schizogony of the vegetative form, 3 and 4 nuclear increase through multiple nuclear division, 5 cell division, 3a division into two of the vegetative forms; 6–12 autogamy within the cyst, 7 first nuclear division, 8 incomplete cell division and chromidium forming, 9 formation of the gamete nuclei from the sexual chromidium, 10 formation of two reduction nuclei, 11 division of the reduced gamete nuclei in motile nuclei (male) and stationary nuclei (female), 12 both the syncaria after fusing of the motile and stationary nuclei, 13 and 14 sporogeny within the cyst, 13 cystwith 8 nuclei; produced by successive mitoses from the two syncaria, 14 bursting of the cyst and freeing of the 8 young amebæ in the intestines of a new host. After Hartmann, from Schaudinn's description (from Kisskalt and Hartmann).

aration shows that the chromidium passes to the periphery of the entoplasm and then into the ectoplasm where it forms a thick network. As the small spherical bodies develop they are seen to be filled with this network of chromidia, but after the refractive brownish membrane forms about them no structure can be made out even in the stained preparations. So the further minute changes in the life cycle at this period are not known. But Schaudinn showed, by experiment, that with these small spherical bodies the infection of the new host is probably brought about. He examined many slides of fæces from one of his amebic

dysentery cases from China, and after finding that they contained no cysts of *Ent. coli*, but only those of *Ent. histolytica*, he washed off the dried fæces with water and fed a certain amount to a young cat whose fæces had been found to contain no amebæ or their cysts. On the third day the cat had bloody diarrhœal fæces containing many forms of the typical *Ent. histolytica*. Twenty-four hours later the cat died, and the autopsy showed typical ulcerative dysentery with the penetration of the amebæ into the epithelia, as Jurgens, and Councilman and Lafleur showed. A cat fed with material from these stools containing only the vegetative forms of the amebæ remained healthy; but the same cat fed some weeks later on material containing many of the small spores came down with typical dysentery six days later and died in two weeks with all the typical symptoms of the disease. This case shows that the cysts and not the vegetative forms in all probability produce the new infection.

The discovery of Viereck and of Hartmann of an apparently new species of ameba causing amebic dysentery in Africa (*Ent. tetragena*) is interesting and makes one realize that the diagnoses of these forms made in their natural habitat should be made with the greatest care. Hartmann's description of the cyst formation and the sexual phenomena of this new form, resemble more the descriptions of these phases in *Ent. coli* than of those in *Ent. histolytica*.

Viability.—The pathogenic amebæ are apt to lose their motility very quickly above or below body heat, while the saprophytic forms remain motile at higher or lower degrees. Though the former lose their motility, they are not all killed by cold. They may still be infective after freezing. Musgrave kept an encysted culture from a dysenteric stool at $-12°$ for 45 days and found it still viable at the end of that time.

A temperature of 60° C. for one hour usually kills encysted cultures of amebæ, according to Strong, but considerable variation has been noted in the degree of temperature necessary to destroy different strains.[1]

Enemata of quinine sulphate and saturated solution of boric acid do not affect amebæ in the intestinal canal, though $\frac{1}{300}$ quinine sulphate added to the stools invariably kills them in ten minutes.

They are also destroyed in stools by weak solution of hydrogen dioxide, potassium permanganate, toloidine blue, and dilute acids.

Luttle found that $\frac{1}{10000}$ hydrochloric acid or $\frac{1}{100}$ silver nitrate check motility, but do not destroy parasites except after prolonged contact. Musgrave and Clegg found that in cultures treated with 1 : 2500 solution of quinine hydrochlorate the parasite quickly encysts, and in from five to eight minutes may break up and disappear; ten minutes later cultures made produced no growth of amebæ, while the bacteria grew well.

[1] An air-dried agar plate culture of "*Amœba coli*" given us by Dr. Calkins who obtained it from the Philippines was viable after three years at room temperature.

Cultures.—We have found that cultures of certain species may be grown with ease on ordinary nutrient agar, as well as upon numerous other nutrient culture media (see p. 536).

Pathogenesis. — Lower Animals.—Just how pathogenic *Ent. coli* is for lower animals cannot be determined, as we have before stated, until a more minute study of the intestinal amebæ is made.

In regard to the amebæ from tropical dysentery (presumably *Ent. histolytica*), it has been shown to be pathogenic to young cats, dogs, and monkeys. The infection may take place in two ways: (1) By feeding material containing the cysts; (2) by rectal inoculations of the vegetative forms. The best work done on dogs is by Harris in 1901, who found that puppies were particularly susceptible after rectal injections of fresh material from human dysentery cases. Morphine was administered before the injection in order to retard peristalsis. The disease developed in two or three days and lasted from four to sixteen days.

The chief symptoms were a bloody diarrhœa and progressive emaciation. The lesions observed in the intestines on post-mortem examinations were a swollen and congested mucosa, over which were scattered numerous small ulcers. In two cases there were liver abscesses.

Microscopically, the mucosa first showed slight exudative and productive inflammation, followed by necrosis and desquamation of the epithelial cells and their basement membrane. At the same time the interglandular tissues beneath became swollen and small hemorrhages occurred. Great numbers of macrophages collected. Ulceration proceeded from above downward. Many amebæ were first seen in and between the epithelial cells, then in the connective tissue at the base or sides of the ulcers. Necrotic and suppurative processes producing varying degrees of suppurative inflammation may complicate the lesions.

The abscesses which form in the liver contain degenerated liver cells, polynuclear leukocytes, red blood cells, and groups of small amebæ.

As controls Harris tried rectal injections of various bacteria, including the Shiga bacillus. All gave negative results, however, and he considered that the amebæ showed their specific action very plainly. Though he did not describe the morphology of the organism from his cases with enough minuteness to identify it with Schaudinn's *histolytica*, he gave enough points to make the inference strong that it is the same species. Whether *Entamœba coli* would produce similar dysentery in young dogs is yet to be proved. As stated above, Schaudinn found that he could produce the typical disease by feeding young cats with cysts of *Ent. histolytica*, but could not get the same results by feeding the vegetative forms.

Musgrave and Clegg injected "pure mixed cultures" of material from cases of clinical amebic dysentery as well as similar cultures of amebæ from various sources into monkeys and produced dysentery. Musgrave fed monkeys with encysted amebæ in bacterial cultures and obtained, in a small percentage of the cases, dysenteric stools and ulcerations in which amebæ were found without their accompanying bacteria. Kartulis, Kruse and Pasquale and Strong

injected into the rectum the contents of liver abscesses containing apparently only the amebæ and produced typical dysentery, with lesions similar to those seen in man.

Strong states that the lower monkey and the orang-outang in the Philippines contract the disease naturally.

In Man.—According to Craig, about 50 per cent. of human beings harbor harmless amebæ in their intestines. Schaudinn states that he found this form of ameba in one-half the cases examined in East Prussia, one-fifth of those in Berlin, and 256 times in 385 examinations in Austria. In order to obtain fresh material for study he infected young cats as did Casagrandi and Barbagallo. He infected himself for a like reason and found that the amebæ remained in his intestines about two months. They remain in the upper and middle parts of the colon where the reaction is alkaline and they produced no pathogenic symptoms.

The disease produced by pathogenic amebæ in man is known as *amebic dysentery* (amebic colitis, amebic enteritis, amebiasis).

Incidence.—The disease occurs endemically in tropical countries. It is particularly prevalent in Egypt, India, and the Philippine Islands. It occurs frequently in parts of South America and southern United States. In northern United States few cases are reported, though Patterson, who in 1909 described three cases (without a description of the amebæ present), and who calls attention to fifteen cases reported as endemic in New York City since 1893, states that this disease is probably more widespread than is generally thought, and that if it were searched for more carefully more cases would be recognized. Patterson adds to his report a bibliography of cases reported as originating in North America. Sporadic cases are found in Russia, Germany, Austria, Italy, and Greece. An occasional small epidemic may occur in the milder climates. Where it is endemic, the largest number of cases occur after the heavy rains have begun in early summer. Males are more frequently attacked, because more exposed to infection. It may occur at all ages, but young adults seem most susceptible. The foreign white race seems to be more susceptible than natives. Unhygienic surroundings are generally a predisposing factor, but in the Philippines all classes are likely to be attacked who do not take continuous and extraordinary precautions in regard to their drinking water.

Symptoms.—The symptoms may be mild or severe. The disease usually runs an irregular course marked by periods of intermission and exacerbation. It may begin acutely with slight fever, griping, tenesmus, and frequent stools. Occasionally, however, the outset is gradual, lasting from a few days to several weeks. The disease is generally chronic, extending over a period of a few weeks or of many years. In the mild form which is usual in children, the general condition may be remarkably good, the only symptoms worth mentioning being the increased number of stools—2 to 6 in twenty-four hours, which contain few to many amebæ. In the severe forms there is a loss of appetite, great emaciation, some fever, acceleration of the pulse, sweating, abdominal pains, and a decided increase of the number of stools

—6 to 20 daily. The stools are more fluid and slimy and may be bloody. They contain amebæ in varying numbers. In very severe forms the stools are watery, filled with blood, mucus, and sometimes sloughs. They vary in numbers from 20 to 50 in twenty-four hours and may contain many amebæ.

The milder forms may change suddenly to the severest, and the severest may suddenly become better and completely recover.

Tissue Changes.—The lesions are chiefly in the large intestines. The walls are thickened in chronic cases, especially the submucosa. There are raised hemispheric areas of hemorrhagic catarrh and of ulceration. The whole of the large intestines may be affected or only more or less circumscribed areas. The amebæ pass between the epithelial cells, generally through small erosions, and they finally reach the submucosa by the lymph channels. Here reproduction takes place and the irritation to the tissue causes œdema and infiltration of small spheroidal cells. This produces small elevations into the lumen of the intestines. The epithelium over these raised areas is finally eroded and then bacteria and intestinal contents help form the succeeding ulcers. The erosions or ulcerations have congested undermined margins, and yellowish-red bases. They vary in size from 2 mm. to about 2 cm. They are round, oval, or irregular in outline. The ulceration usually extends only to the submucosa, but may expose the peritoneum, and large sloughs may be cast off into the lumen of the intestines. Generally the slow inflammatory process in the submucosa leads to great thickening of the intestinal wall.

The processes may be modified in various ways by the action of other microörganisms, especially the bacteria in the fæces. Healing takes place by the formation of connective tissue in the floors and by a gradual covering over with epithelium. In extensive lesions, scars may form.

Peritonitis may occur with the production of an opaque gelatinous fibrinous fluid in which the amebæ may be found.

Abscesses may form in the liver (about 20 per cent. of all cases), less often in the lungs, and only occasionally in the brain and spleen. Amebæ may reach the liver through lymph channels, portal vein, and peritoneal cavity. The other organs are only slightly changed.

Source of Amebæ.—Nothing can yet be said about the exact source of Schaudinn's pathogenic variety, as so few have identified the organism. Strong states that in Manila the greatest source of infection from amebæ is the water supply, that amebæ were cultivated from the water in large numbers in 1902, but no attempt was made to demonstrate their pathogenicity. In 1904, however, Musgrave produced dysentery in a monkey with a culture of a water ameba, though in a few experiments he was unable to infect cats from the amebæ obtained from this monkey. Practically, it is proved that people in Manila avoid being infected with amebæ if they do not drink local water, unless sterilized. Fresh vegetables as well as certain fruits may be sources of infection.

As dilute acids quickly kill the motile amebæ, it is probable that many of those ingested in this form are destroyed in the stomach.

Immunity to the disease may exist. It is supposed that the amebæ as they die produce toxic substances which call forth antibodies, but this has not yet been determined. The necrosis produced in the liver abscesses when bacteria are absent is an indication of the production of necrogenic substances (D. Wills).

Prognosis.—The percentage of deaths in the severe cases is quite

large, especially if accompanied by abscess of the liver. Probably 25 per cent. of all cases are fatal. When treatment is begun early the prognosis is better.

Treatment.—There is no specific curative treatment. Besides rest and diet, high enemata of bisulphate of quinine have been recommended. Harris has gotten good results from hydrogen dioxide enemata diluted from 4 to 8 times with water. About a quart is injected twice daily for a week, then the amount is gradually decreased. Ipecacuanha is highly recommended by Manson, Dock, and others, especially since the introduction of salol-coated pills which allow the remedy to reach the intestines before it is absorbed, so that large doses may be given, without inducing marked nausea and vomiting.

Points in Diagnosis of Amebæ Found in Man.—Examination of stools should be made as quickly as possible after they have been passed and they should be free from urine. The amebæ must be seen motile because, after encystment or death, it is often difficult to distinguish them from other intestinal contents. Bloody mucus or small pieces of necrotic tissue should be examined first as they often contain large numbers of amebæ.

If the movements are solid a dose of salts should be given and the fluid part of the resulting stools examined.

For a differentiation between *Ent. coli* and *Ent. histolytica*, Lesage has recommended the addition of a dilute watery solution of iodine to fluid stools. This causes the characteristic cysts of either form to become noticeable in a few minutes.

Craig differentiates living pathogenic forms from non-pathogenic varieties by the former's: (1) larger size, (2) greenish color, (3) distinct hyaline, refractive ectoplasm, (4) faint nucleus, (5) many vacuoles and red blood cells, (6) marked motility. His differentiation of the stained specimens is given in the table, p. 534.

An absolute diagnosis of liver abscesses can often only be made by an exploratory puncture and the finding of the amebæ. If this is done, the surgeon should be at hand to operate if necessary.

Ent. buccalis is usually found in the thick group of leukocytes and microörganisms collected between the teeth. The amebæ are distinguished from the leukocytes and cell detritus by (1) their large size, (2) their light, highly refractive greenish appearance, (3) their glistening red color in contrast to the yellow-red of the leukocytes when hanging drops are stained with enough of a concentrated solution of neutral red to make them appear pink.

Differential Diagnosis between Amebic and Bacillary Dysentery.—In amebic dysentery (1) the disease is generally chronic; (2) dysentery bacilli are usually not found in fæces; (3) no severe toxic symptoms present; (4) abscess of liver frequent sequela; (5) lesion is in cæcum and descending colon, not in small intestines.

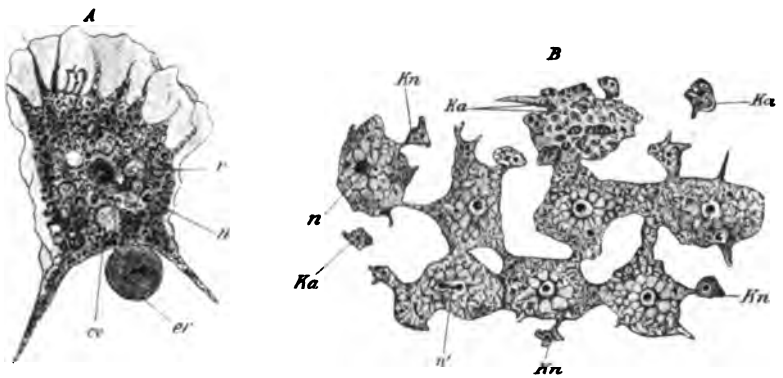
In bacillary dysentery, the finding of the bacilli, and a positive agglutination test, together with the clinical symptoms of intoxication make a certain diagnosis.

AMEBÆ IN OTHER DISEASES.

Baelz found a very large ameba in the bloody urine and in the vagina of a twenty-three-year-old Japanese who was suffering from tuberculosis of the lung. Jürgens, Kartulis, and Posner also reported finding similar amebæ in cases of cystitis and bloody urine.

In the ascitic fluid of a man who had carcinoma of the stomach Leyden found motile cellular elements which Schaudinn first pronounced independent organisms belonging to the rhizopoda and named by him *Leydenia gemmipara* (Fig. 168). Similar organisms were found in the ascitic fluid of a girl who had an abdominal tumor. The

FIG. 168



Leydenia gemmipara (a phase of chlamydophrys stercorea. *A*, single ameba; *B*, plasmodia and budding; *n*, nucleus; *n'*, nucleus dividing; *cv*, contractile vacuole; *v*, vacuole; *er*, red blood cell; *Kn*, buds; *Ka*, ameba developed from bud.

organisms remained motile in the ascitic fluid seven days after removal. The organism possesses a pulsating vacuole and one vesicular nucleus; it divides directly and by budding. The individuals seem readily to fuse (plastogamy). Schaudinn later (1903) considered this organism a phase in the rhizopod chlamydophrys, and decided that it had no pathogenic action.

Amebæ occurring in the mouth have already been noted.

MYCETOZOA.

Introduction.—There is some confusion in regard to placing this group, due to the fact that in it are put many more or less indefinite forms which are difficult to classify. Some forms have both distinct rhizopod and flagellate phases, and they produce simple cysts for reproduction, while others have more plant-like characteristics.

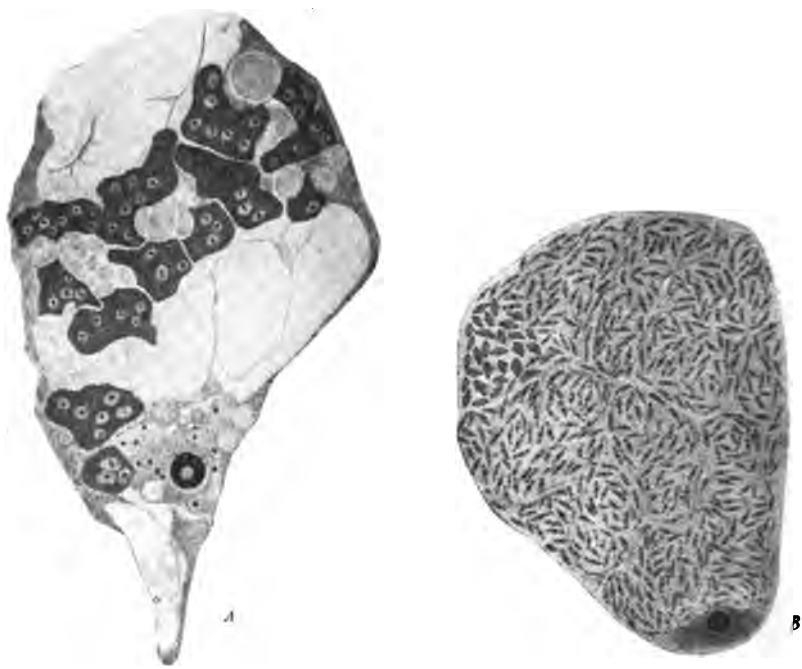
Among the former is placed the *Plasmodiophora brassicæ*, Waronin, of historic interest in medicine because of the claims made from time to time that it or forms related may produce human tumors.

THE PLASMODIOPHORA BRASSICÆ is an intracellular parasite of members of the Cruciferæ, producing large tumors in their roots

("fingers and toes," "club-foot"). When inoculated into experimental animals it produces small granulomata, which finally disappear. The spores taken in by the macrophages under these conditions resemble some of the cell inclusions seen in the human malignant tumors, hence the reason for the belief that under certain conditions they may have an etiologic relationship. At present the idea is abandoned.

The study of the *Plasmodiophora*, however, may be helpful to us in coming to an understanding of the nature of some of the pathogenic protozoa, since it is so closely related to the rhizopoda. Material may

FIG. 169



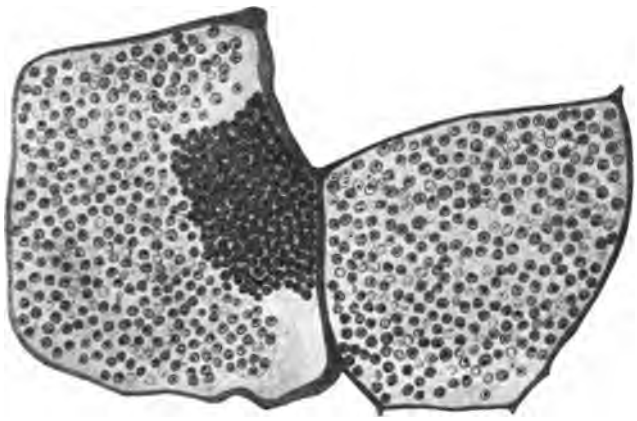
A. Cell of root of cabbage infiltrated with *Plasmodiophora amœbæ*. The amebæ are fusing, forming plasmodia. *B*. Beginning mitotic division of the amebæ. The nucleus of the host cell beneath. (After Nawaschin.)

be more or less easily obtained, and a certain amount of development may be observed in the hanging drop. Much of the life cycle may be satisfactorily demonstrated from sections of cabbage seedlings and the older plants. There are many points, however, in the life history which still need explanation or corroboration.

The Organism.—The roots are supposed to be infected by the flagellated ameboid sporozoites which leave the spore cysts in the moist earth and enter the young rootlets of the seedlings. Here they grow and divide by cell bipartition and by a multiple increase of the nucleus through a primitive karyokinesis. As these forms increase in numbers they are supposed to fuse into a plasmodium due to overcrowding

(Fig. 169 A). Following this fusion there is a simultaneous nuclear division by definite karyokinesis (Fig. 169 B) until the whole host cell is filled with an indefinite mass containing many tiny nuclei, which, according to Prowazek, are sexual nuclei, gametes, that fuse two by two, forming a copula around which a spore wall is produced. Thus,

FIG. 170



Two cells infiltrated with spores of the *Plasmodiophora brassicæ*. (Doflein.)

fertilization by endogamy (sexual union between decendants of the same cell) is accomplished. These small spores fill the dead cell of the host (Fig. 170), and are contained in the soil where they remain until favorable conditions allow the infection of a new host.

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CHAPTER XLI.

FLAGELLATA.

General Characteristics.—Flagellata are protozoa which move, and in some forms feed, by one to several flagella or whip-like processes. If pseudopodia develop, they are transitory.

Generally the flagella arise from the anterior part of the organism, and in motion the larger ones (primary flagella) are directed forward, while smaller ones (secondary flagella) are directed backward, acting as rudders. Certain flagellata possess a modification of their bodies in what is called the undulating membrane, which consists of a fluted protoplasmic process attached along one side of the organism, the free edge of which is prolonged as the flagellum. It has been shown that flagella are not simple protoplasmic processes, but that they have more or less of a framework of elastic fibres as well, hence their power in locomotion can be better understood. Except with special stains, which bring out these fibres, they appear homogeneous.

The flagella arise from some definite place in the cytoplasm, sometimes from a distinctly differentiated chromatic body which has been given various names, such as blepharoplast, centrosome, basal granule, microsome, diplosome, or flagellum root; sometimes directly from the nucleus. The basal granules seems to be derived primarily from the nucleus, and from a physiologic standpoint may be considered as a part of the motor nuclei.

The body of the flagellates is generally more or less elongated and, except in most primitive ones, is fixed in its outline. The latter characteristic is chiefly due to the fact that the organisms usually possess a definite though delicate membrane containing elastic fibrils. The cytoplasm is usually not differentiated into an ento- and ectoplasm. It often contains one to several contractile vacuoles, as well as food vacuoles, and there is frequently a definite opening or cytostom for the entrance of food. There are usually many granules and inclusions of various kinds scattered throughout the cytoplasm, and myoneme striations are seen in some forms. The nucleus, as a rule situated anteriorly, varies much according to different species and to different stages of development.

The flagellata multiply either in the free motile condition or after encystment. In the first case, as a general thing, they divide longitudinally. The basal granules and flagella divide with the nuclei. Multiple division is also observed. In the second case, they may or may not conjugate before they encyst. Then division forms occur in the cyst by a process similar to that in the amebæ.

The sexual cycle varies much in different species. (See Fig. 171.) Isogamy has been noticed between fully grown individuals as well as between smaller forms. The union of different-sized forms, or anisogamy, has also been observed. Also autogamy is not infrequent. Schaudinn claims that certain of the flagellates pathogenic in man require a second host for the development of their sexual cycle.

Natural Habitat.—Flagellates are numerous in foul and stagnant water, along the banks of ponds, lakes, and rivers, in the ocean, in the intestinal contents of various animals, fish especially, and a few in the body fluids of higher animals.

FIG. 171

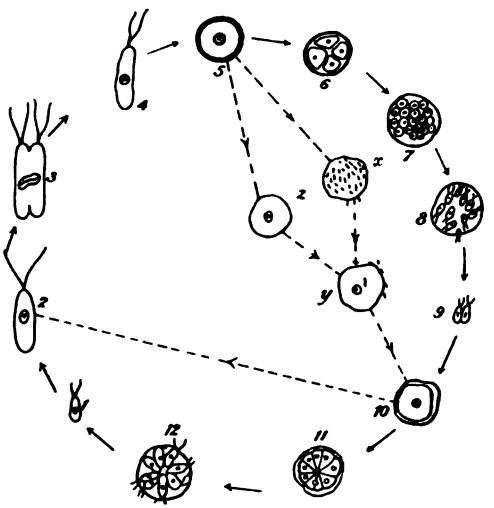


Diagram of variations in life cycle of flagellates: 1, a young flagellate; 2, adult flagellate; 3, longitudinal division of adult free form; 4, daughter flagellate; 5, encystation; 6–8, division into isogametes; *x* and *z*, division into macrogametes and microgametes, characteristic for some forms; 9, conjugation of the isogametes; *y*, conjugation of the macrogametes and microgametes; 10, resting-stage—zygote; 11–12, division into young. (After Doflein.)

Classification.—Following the classification we have adopted, the flagellates parasitic in man are from three orders, the *Monadida*, the *Heteromastigida*, and the *Polymastigida*. The chief differences between these orders are those of size and number of flagella.[1] Under the Monadida are placed the genera *Cercomonas*, *Herpetomonas*, and *Trypanosoma*, with *Spirocheta* as an appendix; *Bodo* is put with the Heteromastigida; and *Trichomonas* and *Lamblia* are classed with the Polymastigida.

Hartmann puts the *Trypanosomata*, with other blood parasites, in a new order, the *Binucleata*, and makes the *Spirocheta* an appendix of this order. According to this arrangement the *Hemosporidia* are taken from the *Sporozoa* and placed with the *Trypanosomata* in this order, the malarial organisms supposedly lose through their parasitism many of the characteristics ascribed to this order.

[1] See classification, p. 529.

Material and Methods for Study.—A number of flagellates (Bodo, for instance, see p. 584) are found in the large intestine of the lower animals. The toad, the grass lizard, and the guinea-pig may contain some interesting forms. As these forms are easily obtained and remain alive a long time outside of the body, they are well fitted for class study.

The fæces are obtained by pressing lightly over the anus of the animal, or if the whole intestinal tract is to be examined, by sacrificing the animal and dissecting out the parts wanted. The material is placed in a clean watch-glass and thinned if necessary with physiologic salt solution. Hanging drops may be made in physiologic salt solution or in such a solution made a little thick by the addition of gelatin in order to retard the motion of the flagellates somewhat so they may be better studied.

Permanent preparations may be made according to directions given on p. 537. As most of the pathogenic members of this group may be difficult to obtain in the living condition at any stated time, they must be studied by students principally in stained smears and sections.

If one can obtain rats infected with *Tr. Lewisi*, others with one or more pathogenic forms; still others with *Spirocheta Obermeieri*, the infecting organisms can be kept alive by frequent reinoculation of the heart's blood, subcutaneously or intraperitoneally into the fresh animal, or cultures may be carried on (see below). But this is an expensive and tiresome work in those laboratories where such work is not being carried on, and generally one must rely on the permanent preparation. In the development in the second host one must also study the stained specimens in the great majority of instances.

The fresh specimens of blood are obtained from the tail tip of the rat, or the ear of the dog; they may be examined after dilution with physiologic salt solution in the hanging drop, or in a drop spread under a cover-glass and ringed with vaselin. For permanent preparations films of the blood are spread, fixed, and stained in the usual way; Giemsa's method of staining (p. 624) is very satisfactory.

For section work of the various organs the fixatives and methods given on page 521 may be used. Special methods are given under each organism.

Artificial Cultures of Blood Flagellates.—These, according to Novy and MacNeal, may be made on a culture medium consisting of a mixture of ordinary nutrient agar with variable amounts of fresh defibrinated rabbit or rat blood. The best all around results are obtained with equal parts of blood and agar. The agar is melted and cooled to 50° C., then the blood is added and thoroughly mixed. The tubes are inclined until the medium stiffens, when they should be inoculated at once with blood or other infected material containing living trypanosomes. The surface of the medium should be very moist, so water of condensation may form. Generally evidence of growth may be observed in three or four days.

CERCOMONAS.

The members of this genus are round or oval flagellates with a long anterior flagellum and a more or less pointed posterior one which is sometimes ameboid. The vesicular nucleus is situated anteriorly, and lying near it are one or two contractile vacuoles. Divison into two daughter forms has been observed.

A number of cercomonada, none of them well studied, have been observed in different animals as well as in man.

Cercomonas hominis (Davaine, 1854) was observed in the dejections of a cholera patient by Davaine. The body is 10μ to 12μ long and

pear-shaped, pointed posteriorly. The flagellum is twice as long as the body. The nucleus is difficult to see. Davaine also reported a smaller form in the stools of a typhoid patient. Other observers have noticed similar forms in human stools, some associated with "*Amœba coli.*" Similar forms have been seen also in an echinococcus cyst of the liver, in the sputum from a case of lung gangrene, in the exudate of a hydropneumothorax, and a few times in the urine. They are all probably harmless invaders.

HERPETOMONAS AND CRITHIDIA.

Certain flagellates found in the digestive tract of mosquitoes, flies, and other insects are very similar to the trypanosomata. Among them two distinct types have been recognized, *Herpetomonas* and *Crithidia*, the main differences between them being (1) the large size of the adult monadian form of the former as compared with the latter, and (2) a rudimentary undulating membrane in the latter. The distinctions between these two genera and the genus *Trypanosoma* which have been recognized are: (1) the former contain no undulating membrane or only a rudimentary one, and (2) their centrosome or blepharoplast usually lies at the side of, or anterior to, the nucleus instead of posterior to it, as in *Trypanosoma.*

These distinctions, Novy claims, disappear in the cultural forms of the three genera, when all show a rudimentary undulating membrane and an anterior blepharoplast; he therefore considers them all one genus, *Trypanosoma.* But most protozoölogists do not agree with him. His caution, however, in regard to confusing these insect flagellates with developmental stages of vertebrate blood parasites should be remembered.

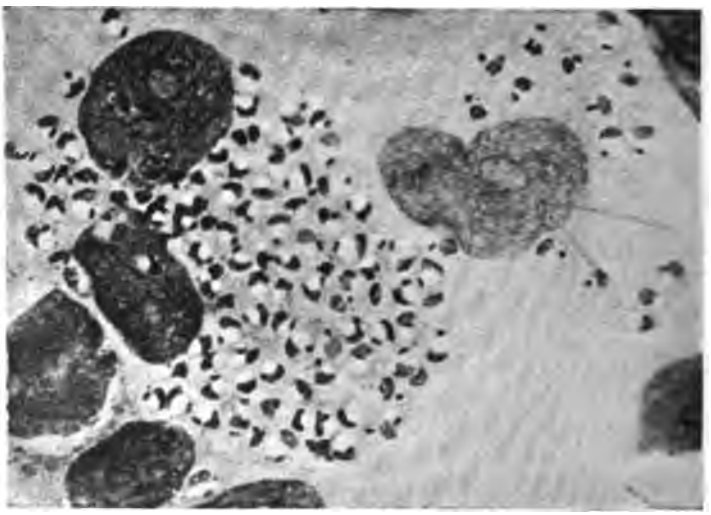
From the recent work done on the Leishman-Donovan bodies found in kala-azar it seems probable that they are closely related to this group of flagellates. Indeed, the latest conclusion reached in regard to the classification of these organisms is that they belong to the genus Herpetomonas. Babesia (Piroplasma), on the other hand, which was thought earlier to be closely related to these genera, now, on account of lack of corroborative studies, is put as it originally was under the hæmosporidia.

HERPETOMONAS DONOVANI (LEISHMANIA DONOVANI, LEISHMAN-DONOVAN BODIES) AND ALLIES.

Certain fevers of severe malarial-like types known in different sections of the tropics by different names (dum-dum fever, cachexial malaria, kala-azar) have recently been shown to have a causal relationship by the finding of similar protozoön-like bodies in the lesions. These bodies were first minutely described by Leishman in 1903 as being present in certain cells in the spleen of cases called by him dum-dum fever, occurring in India. He considered them as possibly trypanosomes, but did not name them. Later in the same year Donovan described similar bodies in cases of what he called malarial cachexia. The

bodies were first called the Leishman-Donovan bodies, then Laveran and Mesnil who examined Donovan's preparations and considered the organisms similar to those causing Texas fever in cattle, called them *Piroplasma Donovani*. Ross, however, thought they constituted a distinct genus and named them *Leishmania donovani*, by which name they are still known. But there is little doubt, through the work of Rogers and of Patton, of their belonging to the genus Herpetomonas. They have since been found in different parts of India, in China, Tunis, Algiers, Arabia, and Egypt, and Wright in this country has reported in a case of Delhi boil from an Armenian immigrant, bodies which, according to his excellent photographs and description, must be identi-

FIG. 172



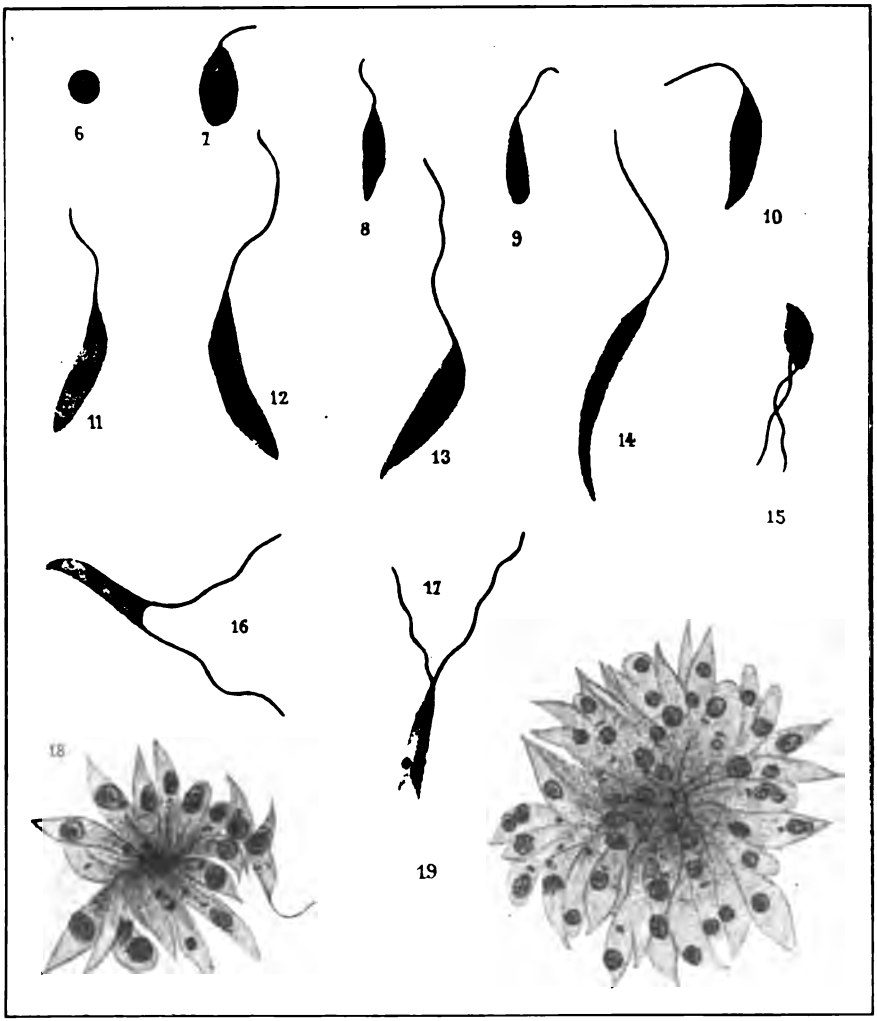
Protozoa in a case of tropical ulcer. Smear preparation from the lesion stained with Wright's Romanowsky blood-staining fluid. The ring-like bodies with white central portions and containing a larger and a smaller dark mass are the microörganisms. The dark masses in the bodies are stained a lilac color, while the peripheral portions of the bodies in typical instances are stained a pale robin's-egg blue. The very large dark masses are nuclei of cells of the lesion. × 1500 approximately. (After Wright.)

cal with, or very closely related to, Leishman's bodies. On account of the different pathologic conditions in which they are found, they are considered by some a different species, *Herpetomonas tropica*, Wright. The form found in infantile splenomegaly may be considered another species, with the name *Herpetomonas infantile*, until further study. Darling has recently described an organism resembling that of kala-azar found in a fatal disease of tropical America. Though the organism, he says, resembles *H. Donovani*, he thinks it has enough points of difference to be placed in a different genus; therefore he gives it the name *Histoplasma capsulatum*, and calls the disease histoplasmosis. He says it differs from H. Donovani in the form and arrangement of its chromatin nucleus and in not possessing a chro-

matin rod. It has a refractile achromatic capsule. As he has not yet grown the organism or made studies on its intermediary host, it is too early to determine whether his generic name will hold.

The bodies have been found in large endothelioid cells in the spleen,

FIG. 173



Cultural forms of *H. infantum*, showing the flagellate type. (After Nicolle).

liver, mesenteric glands, bone-marrow, kidney, lungs, testes, skin, ulcers in intestines, and in the polynuclear leukocytes in the peripheral blood. In this last situation they are only found in appreciable numbers in advanced cases.

Morphology.—The bodies are circular to elliptical in shape, from 2μ to 4μ in diameter, and contain a double nucleus, a large oval one at one part of the periphery and a small circular or rod-shaped one near or at the opposite part of the periphery. This smaller body stains more intensely than the larger one, while the cytoplasm of the parasite stains very dimly, sometimes showing only a faint peripheral rim. Any nuclear and cytoplasmic staining methods will bring out these points in Zenker-fixed material. Smears stain well by Wright or the Nocht-Romanowsky methods. The large cells containing the parasites are supposed by Christophers to be the endothelial cells from the finest capillaries. Donovan states that he found small forms in the red blood cells in the peripheral circulation when the temperature was above 103°, and Rogers has grown abundant pure cultures of the bodies in a slightly acid citrated blood medium at 20° to 22° C. Nicolle and later Novy have shown that *H. infantum* is pathogenic for dogs and that cultures may be obtained with ease from the infected animals (Fig. 173).

Rogers and Patton have shown that the bedbug transmits the disease, and Patton has demonstrated the development of the organism up to the fully flagellated stage in the gut of this insect.

Effect on Human Host.—The symptoms, in the cases of general infection are: (1) very much enlarged spleen and less enlarged liver; (2) progressive anæmia with peculiar earthy pallor of skin, progressive emaciation, and muscular atrophy; (3) long-continued, irregularly remittent and intermittent fever (97° to 104°); (4) hemorrhages, such as epistaxis, bleeding from gums into subcutaneous tissue, producing purpuric eruption; (5) transitory œdemas of various regions. There are often complications, such as congestion of lungs, dysentery, and cancrum oris. The blood count shows practically no loss of hæmoglobin, but there is a decrease in the leukocytes, principally polynuclears, giving a relative increase of mononuclears.

Negative points which help in the diagnosis are: absence of malaria, no typhoid or Malta fever reaction, resistance to medication, quinine, as a rule having no effect, though in early cases, and with large continued doses a few good results have been reported. Splenic puncture with the finding of Leishman-Donovan bodies makes the diagnosis certain.

The duration of the disease is from a few months to several years. The percentage of deaths is great; in some forms of the disease, at the height of an epidemic, it may reach 98 per cent.

Segregation and perfect cleanliness, especially in regard to bedbugs, are recommended as the best means of eradicating the disease.

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CHAPTER XLII.

TRYPANOSOMA.

Introduction.—The name trypanosoma (boring animal) was given by Gruby in 1843 to certain free-swimming hæmoflagellates found by him in the blood of frogs. Much later similar flagellates were found in the blood-plasma of many different species of vertebrates and in the intestinal tract of several blood-sucking invertebrates.

Typical trypanosomes are characterized (Fig. 175, p. 562) by a comparatively long, spirally-twisted body, along one side of which is attached an undulating membrane having a cord-like edge that is continued forward as a free whip (flagellum). The flagellum arises near the posterior end of the organism in a small granule, called the blepharoplast which lies near, or may be merged with, a larger chromatin mass, called the kineto-nucleus because of its control over the motor apparatus.

The nuclear apparatus consists of a tropho-nucleus with an intranuclear centrosome, and of the above-mentioned kineto-nucleus and blepharoplast which last functions as a centrosome for the kineto-nucleus. The tropho-nucleus is usually situated nearer the flagellar end; it is granular, thick, and egg-shaped, but varies somewhat in size and shape.

The cytoplasm is faintly alveolar or granular, varying with age, environment, and possibly species. Reproduction occurs usually by longitudinal, occasionally by multiple division. The life cycle is not well known. Though transmission occurs through the bites of various invertebrates, notably flies, no definite sexual changes have been proved to take place in the intestines of these intermediate hosts. That an intermediate host is not necessary for the continued life of at least one species of trypanosome seems to be proved by the fact of direct transmission of *T. equiperdum* from horse to horse through coitus.

Pathogenic Forms.—About 60 species of trypanosoma have been described, but of these only a few are reported as distinctly pathogenic, and two of these are known to be pathogenic for man. All these are found in tropical countries. The accompanying table gives a list of the better-known pathogenic forms with their hosts and with the diseases produced by them. Though only slightly pathogenic, *T. lewisi* is included among them, because of its similarity to the more pathogenic forms and because of the ease with which it may be obtained and studied.

TABLE OF PATHOGENIC TRYPANOSOMES (INCLUDING T. LEWISI)

Species, name	When discovered	Vertebrate host	Disease produced	How transmitted (invertebrate host)	Dimensions in μ (including flagella and undul. memb.)	Other characteristics
T. lewisi, Kent ...	1878	Rats.	Occasional epidemics.	Louse (Hæmatopinus spinulosus).	8–30 × 1.5–3	Narrow and pointed, aflagellar extremity, trophonucleus in ant. third of body.
T. evansi, Steel.....	1880	Horses, mules, cattle, camels, elephants, buffaloes.	Surra	Stomoxys (flies).	20–30 × 1–2	
T. brucei, Plimmer and Bradford.	1894	Horses and cattle.	Nagana	Tsetse flies, esp. glossina morsitans.	26–35 × 1.5–2.5	Post. end usually bluntly rounded.
T. equiperdum, Doflein.	1896	Horses, asses.	Dourine.	By coitus.	24–28 ×	
T. dimorphon, Dutton and Todd.	1902	Horses	Trypanosomiasis in Senegambia	(?)	13–30 ×	Long and short form with protoplasm reaching to top of flagellum.
T. theileri, Bruce...	1902	Cattle.	Gall sickness.	Hippobosca rufipes	60–70 ×	The largest form—a kineto-nucleus often near midbody.
T. equinum, Voges..	1902	Horses, cattle.	Mal de Caderas.	Hydrochœrus capybara.	22–25 × 1.5–2	Kineto-nucleus very minute.
T. gambiense, Dutton.	1904	Man.	Trypanosomiasis (Sleeping sickness)	Glossina palpalis.	17–23 × 1.5–2	The smallest form—post. end bluntly conical.
T. cruzi, Chagas....	1909	Man and monkeys.	Trypanosomiasis (Sleeping sickness)	Conorrhinus.	Small.	Large blepharoplast (kineto-nucleus).

Historical Note.—The first species of trypanosome studied with any degree of fullness is the comparatively non-virulent *T. lewisi*. It was probably first seen in the blood of the rat in 1845, but was not well described until 1879, when Lewis studied it more fully. Since then it has been studied by many observers, especially by Kempner and Rabinowitsch, Wasielewski and Senn, Jürgens, Laveran and Mesnil, Novy and MacNeal, Prowazek and Moore, Breinl and Hindle.

The first of the more pathogenic trypanosomes was discovered by Evans in the blood of East Indian horses suffering from surra, but it was not well studied until 1893, when Lingard's important work on surra led, in a way, to all the subsequent work on diseases caused by trypanosomes. The next year a trypanosome was discovered by Bruce in the blood of horses and cattle suffering from nagana in Zululand and other parts of Africa. Bruce further demonstrated the important fact that the disease was transmitted by the bites of flies, the tsetse flies (*Glossina morsitans*). Announcements of other pathogenic trypanosomes in different parts of the tropics quickly followed. In 1896 Rouget found that dourine, a disease of equines in Algiers and South Africa, was caused by a trypanosome (T. equiperdum). Then the South African disease of horses, called mal de Caderas, was shown by Voges to be due to a similar flagellate, while in 1902, Theiler found a variety of trypanosome in the blood of cattle in the Transvaal suffering from the disease called galziekte, or gall-sickness. The number of trypanosomes found in the tropics is constantly increasing—both pathogenic and non-pathogenic forms.

Man was thought to be immune to trypanosomes until recently. Then a few isolated cases of infection were reported before the important discovery was made that trypanosomes are the specific cause of a definite disease known as sleeping sickness, which occurs chiefly in the African negro.

In 1898 Nepvieu reported having found trypanosomes in the blood of 6 out of more than 200 cases of human beings examined for malarial organisms and in a seventh case which was apparently in good health.

The eighth case is reported by Dutton in 1901. This case was a European who had lived some years in West Africa. The principal symptoms were gradual wasting and weakness; irregular temperature, never very high and of a relapsing type; local œdemas, congested areas of the skin, enlargement of the spleen, and constant increased frequency of pulse and respiration. It ended fatally after one year and eight months. The chronic character was repeated in animals. Some white rats were refractory; others died in two to three months. In monkeys (*Macacus rhesus*) it was fatal in about two months. Dogs were unaffected. This trypanosome was distinctly smaller than species described in lower animals, and there was little doubt of its being a distinct species. Dutton also found trypanosomes in the blood of 1 out of 150 apparently healthy Gambian children examined by him.

The tenth case is published by Manson in 1902. This was a missionary's wife who had resided on the upper Congo for a year. She presented the same group of symptoms as Dutton's case, and after repeated examinations trypanosomes were found in her blood. Manson soon after published a similar case. Broeden published two more cases, and Baker three.

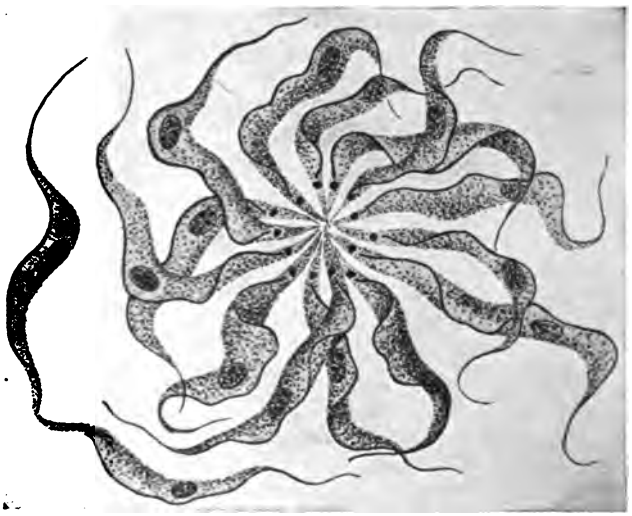
In 1904 Castellani stated that the cause of sleeping sickness of the negro is a trypanosome. He found trypanosomes in the centrifugalized cerebro-spinal fluid of 20 out of 34 cases of this disease. His work has been corroborated by Bruce, Nabarro, Greig, and others. Bruce found trypanosomes in the fluid obtained by lumbar puncture in all of the 38 cases examined and in 12 out of 13 cases in the blood. The trypanosomes found in these cases resemble those already found in other human beings, and probably belong to the same species; they are, therefore, included under the same name, *Trypanosoma gambiense*, Dutton.

Chagas, in 1909, states that a trypanosome which he had discovered in a small monkey (*Callithrix hapalepenecellata*) and called *T. cruzi*, is the cause

of human infection in Rio de Janeiro. It is carried by a hemiptera, genus, conorrhinus. The flagellate is small with a large blepharoplast (kinetonucleus). It grows on blood agar readily and infects laboratory animals easily. Chagas reports developmental forms in the monkey's lung and in the gut of the fly.

Comparative Characteristics of the Different Species.—The form changes of the same species in the same host are so varied that few have been found absolutely characteristic of a single species, and, as physiologic properties are not used in species classification, we cannot be sure that all of the organisms in this group described as separate species are so until more of the complete life histories are known; until

FIG. 174.



Agglutination of *Trypanosoma Lewisi*. (Laveran and Mesnil.)

this time each new form found with distinct physiologic properties, though apparently morphologically similar to others, may expediently be considered a new species.

Morphology.—Size.—The variations recorded in the *dimensions* of the eight species we are considering may be seen by glancing at the above table. The trypanosome pathogenic for man (*T. gambiense*) has the smallest average size of the group. With the exception of *T. theileri*, which is much larger than any other of these eight forms, the variations in size of the different species are not so marked as they are on the same species under different conditions.

Shape.—In shape, though all follow the type, each species varies greatly according to conditions of growth and multiplication. At times they may be slender and worm-like, at others they may be so short and thick as to be almost round. *T. lewisi* has the posterior (aflagellar) end often thinner and more pointed than the other species. *T. evansi*

PLATE II

Various Spirochetes (after Muhlins) and Blood Parasites. (All Giemsa stain except 7, which is by Levaditi's method.)

1. Trypanosoma Lewisi. 2. Spirocheta balbianii. 3. Spirocheta from mouth: *a*, Spirocheta buccalis; *b*, middle form; *c*, Spirocheta dentium. 4. Spirocheta dentium, pure culture. 5. Spirochetæ with Vincent's fusiform bacillus. 6. Spirocheta pallida from ulcus durum. 7. Spirocheta pallida, from liver section. 8. Spirocheta gallinarum. 9. Spirocheta from esophagus carcinoma. 10. Spirocheta (?) or Spirilla (?) in mouse blood. 11. Spirocheta from lung gangrene 12 Estivo-autumnal parasite: *a*, ring form; *b*, macrogametocyte. 13. Quartan parasite: *a*, half-grown organism; *b*, full-grown organism. 14. Prioplasma bigeminum, showing various stages in division within red blood cell.

is generally a little longer and thinner than *T. lewisi*, while *T. brucei* has a more rounded aflagellar end than either, and is generally wider.

The cytoplasm differs slightly in the different forms. *T. lewisi* is relatively free from chromatoid granules, while *T. brucei* has usually many. Myoneme fibrils have been demonstrated in some species and probably all contain them. An oval vacuole has been seen in some species.

The nuclear apparatus is essentially similar in all forms. The two nuclei (tropho- and kinetonucleus) vary somewhat in position and size in the different species and at different stages in the same species. In *T. theileri* and in young forms of *T. lewisi*, both nuclei lie close together near the centre of the organism. In *T. lewisi* the tropho-nucleus is situated more anteriorly than in the other species.

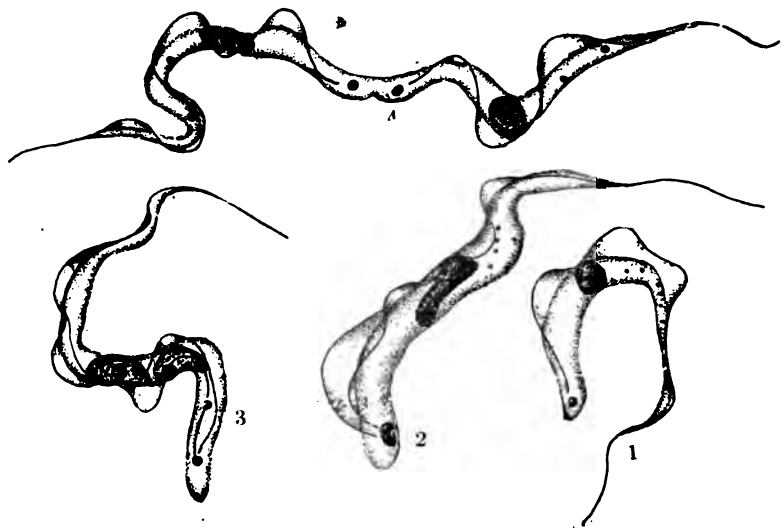
Many variations from the type forms are seen. Some are no doubt degeneration and involution forms. *Three* forms, however, which are more or less constantly seen in all the species have been interpreted as definite phases in the life cycle. These forms were first described by Schaudinn in *T. noctuæ*, and were interpreted by him and since then by others as male, female, and indifferent form. The male cells are smaller, more hyaline, and more free from granules than the female. The nucleus of each sexual cell rids itself of male and female chromatin, respectively. The indifferent cell, on the contrary, has a complete nucleus. Opinions differ as to Schaudinn's interpretation being the correct one. More research is needed before we can arrive at a definite conclusion.

Motility.—The first thing noticed on examining a fresh hanging drop of blood at a magnification of 100 to 300 diameters is active movements of the red blood corpuscles in certain areas, and, on carefully focusing over one of these areas, the rapidly wriggling worm-like organism may be seen. As the movements become slower, the flagellum may be seen swaying from side to side and the wave-like movements of the undulating membrane are quite discernible. Movement is two-fold: (1) progression with an auger-like motion effected by the undulating membrane assisted by the flagellum; (2) contractions of the body assisted no doubt by myoneme-like structures. Relatively, *T. lewisi* is most active and *T. brucei* least. Motility soon ceases outside of the body, continuing longer if the organism has been kept in the ice-box than at higher temperatures.

Reproduction.—The usual and probably the universal method of multiplication is binary longitudinal fission (Fig. 175). In *T. lewisi* a rosette-like segmentation has also been observed. Longitudinal fission begins usually with division of the kineto-nucleus, then of the flagellum, and last of the tropho-nucleus and cytoplasm; but this order of division seems to be quite variable. The flagellum often appears to be dividing first, and probably division always starts with the centrosome-like basal granule of the flagellum. In some cases a new flagellum seems to be formed instead of division of the old one. The details of division have not been frequently studied, but it is probable that both nuclei

divide by a primitive mitosis. During division the kineto-nucleus generally moves near the tropho-nucleus. The cytoplasm divides last, beginning usually at the flagellar end. Generally this fission is equal, but occasionally the daughter trypanosomes may be quite unequal in size. This is notably the case in division of *T. lewisi* where the cytoplasm may divide so unequally that the process may be compared to budding. The resulting small parasites have at first no undulating membrane, hence they resemble somewhat Herpetomonas. These young forms may divide several times in succession, producing smaller

FIG. 175



Trypanosoma gambiense in longitudinal division. (From Calkins. Preparation by F. W. Bahstack.)

and smaller fusiform parasites. As a result, some forms are so small that they can only be seen when agglomerated or in motion (Schaudinn).

The Life Cycle of Trypanosomes.—The question as to whether trypanosomes undergo phases of development in their invertebrate hosts has been widely studied, especially since Schaudinn's observations on *Hæmoproteus noctuæ* indicated a complex life cycle of the hæmoflagellates and their close relationship to the hæmosporidia. Schaudinn's work still holds, according to many observers, but some, notably Novy and his co-workers, insist that Schaudinn did not give evidence of having sufficiently guarded against the error of mixing up the life histories of distinct protozoa. Schaudinn claimed that the intracellular hæmoproteus of the owl classed with the hemosporidia is only a stage in the development of *Trypanosoma* (trypanomorpha) *noctuæ*, which is transmitted to owls by the mosquito *Culex pipiens*, in whose gut it undergoes sexual changes. Novy and others claim that Schaudinn's mosquito phases are forms of mosquito flagellates and not of the bird trypanosome.

Encysted forms of some species have been seen by certain observers in the fly carriers (Minchin, Laveran and Mesnil, and others). Other species, however, seem to undergo no important change in the fly, so the whole question is waiting for further research.

That the different forms of trypanosomiasis (with the single exception of dourine) are transmitted by the bites of flies is a fact. Bruce (1894) first showed that *T. brucei* was conveyed by the fly *Glossina morsitans*. Since then other varieties of flies also have been shown to spread the disease. *Glossina palpalis* (see Fig. 176) is supposed to be the chief agent in transmitting human trypanosomiasis. These flies bite by day and in full moonlight. The infectivity of the insects lasts for about forty-eight hours after they have bitten a sick animal. Bruce found living trypanosomes in the proboscides of the flies at the end of that time. Up to one hundred and eighteen hours they were found in the flies' stomachs, but after one hundred and forty hours the stomachs were empty, and what appeared to be dead parasites were found in the excreta.

Cultivation.—Novy and MacNeal were the first (1903) to cultivate trypanosomes in the test-tube. They have grown *T. lewisi* through about 100 culture generations extending over several years. At the end of this time the parasites were as virulent as at the beginning. The culture medium used in their work was ordinary nutrient agar containing variable amounts of fresh defibrinated or laked rabbit or rat blood. The best results were obtained with a mixture of equal parts of blood and agar. At room temperature the growth is slower but surer than in the thermostat. A culture at room temperature retains its vitality for months; thus in one case the trypanosomes were alive after three hundred and six days. Novy and MacNeal also cultivated *in vitro T. brucei, T. evansi,* and various bird trypanosomes. The latter they found especially easy to cultivate, while the former are much more exacting in their requirements than is the *T. lewisi*. These investigators state that the cultural characteristics are such as to enable perfect differentiation between *T. Brucei* and *T. Lewisi*. For in cultures *T. brucei* has characteristic granules, *T. lewisi* has none; the *T. brucei* shows little variation in size (15μ to 17μ in length), *T. lewisi* varies so much (1μ to 60μ long) that there are forms small enough to pass a Berkefeld filter; *T. brucei* has a slow, wriggling motion, *T. lewisi* moves with great rapidity and in an almost straight line; and finally *T. brucei* forms small, irregular colonies, while *T. lewisi* forms large, symmetrical ones.

The great majority of trypanosomes experimented with have been found by various workers to be cultivatable, with more or less ease. *T. gambiense,* however, the cause of human trypanosomiasis, has so far resisted artificial culture methods.

Effect on Vertebrate Host (Pathogenesis).—Lower Animals.—Many of the lower vertebrates have become, through mutual toleration, natural hosts of the trypanosome. It is probable that each pathogenic trypanosome has an indigenous wild animal as natural host and that

in this way the supply to strange mammals coming into the vicinity is kept up. These strange animals, being unaccustomed to the native trypanosomes, succumb to the infection.

In general the descriptions given of the symptomatology of trypanosomiasis in various animals show a great similarity, though there is much variation in individual cases. The average clinical picture, according to Musgrave and Clegg, is as follows: After an incubation period which varies in the same class of animals and in those of different species, as well as with the conditions of infection, and during which the animal remains perfectly well, the first symptom to be noticed is a rise of temperature, for some days a remittent or intermittent fever may be the only evidence of illness. Later on the animal becomes somewhat stupid; watery, catarrhal discharges from the nose and eyes appear; the hair becomes roughened and falls out in places and the peripheral lymph nodes are enlarged. Finally the catarrhal discharges become more profuse and the secretions more tenacious and even purulent: marked emaciation develops; œdema of the genitals and dependent parts appears; a staggering gait, particularly of the hind parts, comes on, in some forms passing on to paralysis. This is followed by death. There may be various ecchymoses and skin eruptions. Parasites are found in the blood more or less regularly after the appearance of the fever. They are often more numerous in the enlarged lymph nodes and in the bloody œdematous areas than in the general circulation.

The autopsy generally shows anæmia, an enlarged spleen with hypertrophied follicles, more or less gelatinous material in the adipose tissue, the liver slightly enlarged, a small amount of serous exudate in serous cavities, œdematous condition, and small hemorrhages in various tissues.

The duration varies from a few days to many months. The prognosis seems to be influenced to a certain extent by the species of host. It is probably always fatal in horses. Some cattle recover. The cause of death is possibly a toxic substance, though no definite toxin has been isolated. Mechanical disturbances (emboli, etc.) also probably play a part in producing death.

Man.—*Sleeping sickness*, or *human trypanosomiasis*, is a disease of the negro, endemic in certain regions of equatorial Africa. Neither age nor sex are predisposing factors, but occupation and social position seem to have a marked influence, the great majority of cases occurring among very poor field workers. As these workers are all negroes, the question of the influence of race cannot be determined. The white race, however, is not immune, as has been shown by the cases quoted above.

In places where most of the cases occur, a fly belonging to the species glossina (*Glossina palpalis*, see Fig. 176) is very abundant; in places where this fly is not found no cases occur. Hence, it is highly probable that, as in the trypanosomiasis of the lower animals, the contagion is spread by a biting insect.

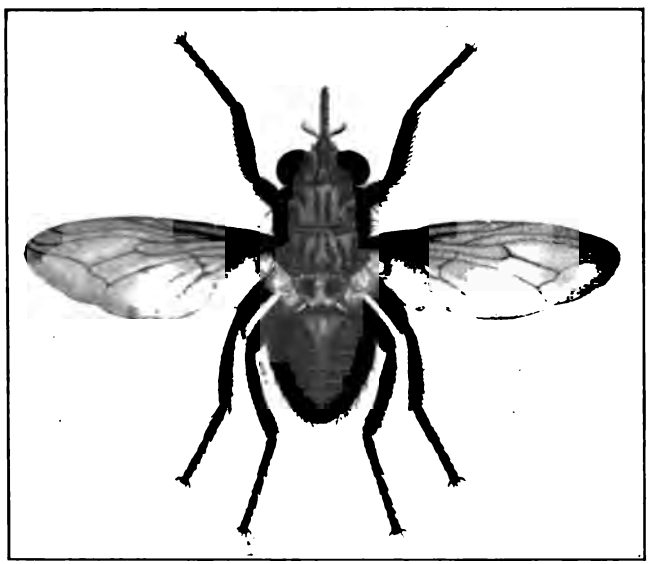
Symptoms.—The course of the disease is very insidious, as the trypanosomes may exist in the blood for a long time before entering and growing in the cerebrospinal fluid and causing the characteristic symptoms of sleeping sickness. Therefore, the symptoms may be divided into two stages. In the first stage there is only an irregular fever with enlargement of the peripheral lymph nodes. In the second stage the fever becomes hectic, the

pulse is constantly increased; there are neuralgic pains, partial œdemas and erythemas, trembling of the muscles, gradually increasing weakness, emaciation, and lethargy. The somnolence increases until a comatose condition is developed and death occurs. In the second stage trypanosomes are always found in the cerebrospinal fluid. Throughout the disease they are usually found in small numbers in the blood.

Duration.—The first stage may last for several years; the second, from four to eight months. The percentage of deaths in cases reaching the second stage is 100. Whether some in the first stage recover is not yet certain.

T. gambiense, the chief trypanosome pathogenic for human beings, is irregularly pathogenic for some monkeys (*Macacus rhesus* and others), for dogs,

FIG. 176



Glossina palpalis, carrier of human trypanosomiasis. ×4 (Kolle und Wassermann).

cats, and rats. It is less pathogenic for mice, guinea-pigs, rabbits, and horses. Cattle and swine seem to be refractory.

Pathological Changes.—Congestion of the meninges; increased quantity of cerebrospinal fluid; hypertrophy of spleen, liver, and lymphatic ganglia; diminished hæmoglobin and number of red cells; number of leukocytes about normal, but a relative increase of eosinophiles, mast cells, and lymphocytes. Enlargement of the superficial lymph nodes has been noted as an early symptom and has thus been made use of in diagnosis. Dutton and Todd found that 91 per cent. of natives in the Congo Free State, who had posterior cervical glands enlarged, showed trypanosomes in the punctured gland juice.

Diagnosis of Trypanosomiasis in General.—This should be made as early as possible in order to prevent the spread of the disease. An early positive diagnosis can only be made by the determination of the peripheral infection. This is done in two ways: first, by the micro-

scopic examination of a hanging drop of freshly drawn blood, or tissue from enlarged peripheral lymph nodes; second, by animal inoculation of the blood or other tissue. In the microscopic examination it may be necessary to examine the blood of the suspected animal for several days in succession. The parasites are rarely absent in the early stages in domestic animals for more than a few days at a time, while in man the time may be much longer.

Methods of Examination.—BLOOD.—If the direct examination of the blood is negative, 10 c.c. should be withdrawn from the vein, and after adding a tenth of its volume of citrate of sodium it should be centrifuged for ten minutes, and the sediment examined in hanging drop and in smear. The great majority of the parasites will be found collected with the white cells in the thin white layers which may easily removed with a fine pipette. If only a small amount of blood can be obtained, the tiny tubes recommended by Wright in his opsonin work (p. 183) may be used.

CEREBROSPINAL FLUID.—Ten c.c. of the fluid withdrawn by lumbar puncture should be centrifuged for fifteen minutes and the deposit should be examined under 150 to 200 diameter magnification.

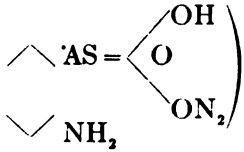
THE INOCULATION TEST.—If the trypanosomes cannot be found by the above methods, animal experiment should always be made. Monkeys, if possible, should be used, or if monkeys cannot be obtained, dogs or rats may be used. A few drops to 1 c.c. of the blood or other tissue from the suspected animal should be inoculated intraperitoneally or subcutaneously.

Blood smears may be stained by any modification of the Romanowsky method. Giemsa's method (p. 624) gives good results.

Prophylaxis.—The disease is readily controlled by preventive measures. There should be strict quarantine regulations governing the importation of animals. When the disease has once appeared, the following general measures should be taken: 1. Suspected animals should be isolated. 2. All infected animals should be destroyed. 3. As far as possible, all biting insects should be destroyed. 4. The bodies of infected animals should be protected from biting insects for at least twenty-four hours after death. 5. Susceptible animals should, if possible, be made immune.

Treatment.—The whole question of treatment is still in the experimental stage. The chronic course of the disease with relapses often after long intervals makes it impossible, especially in cases of human trypanosomiasis, to come quickly to a conclusion in regard to the efficacy of any drug. Many drugs have been found to possess trypanocidal properties. They may be classified according to Breinl and Nierenstein into three groups: 1. Compounds containing arsenic in inorganic form, as sodium arsenate. 2. Those containing organic radicals, as the amine group in atoxyl and allied compounds and in certain coloring substances of the diazo type (trypanred, parafuchsin, tryparosan, etc.) introduced by Ehrlich. 3. Antimony in form of sodium antimonyl tartrate and isomers.

Atoxyl (p-amino-phenyl-arsenic acid,



introduced by Thomas (1905) and used first by Thomas and Breinl in treatment of experimental trypanosomiasis, has proved to be the drug above all others to have a beneficial effect in the different forms of this disease. Much experimental work has been done on the different phases of treatment by atoxyl and its allies, in the course of which some very interesting facts relating to chemo-therapeutics have been demonstrated. Ehrlich has added to his "side-chain theory," while others have advanced quite different views in regard to the action of this group. The chief facts are the following:

1. Atoxyl does not act *in vitro* unless mixed with an oxidizing substance.

2. After the first few treatments with any of these drugs trypanosomes may become resistant to the drug. This resistance is more or less specific for all members of the group to which the drug used belongs. There are a few exceptions, *e. g.*, an atoxyl-resisting strain may still be influenced by arseno-phenyl-glycine or by orsudan.

This acquired resistance lasts for some time in the species of animal used, but may be quickly lost if the resisting trypanosomes are inoculated into another species.

3. The time of the reappearance of parasites after a discontinuation of treatment is more or less regular. With *T. gambiense*, in rats and monkeys, the period is generally 50 to 60 days. With *T. brucei*, in rats, guinea-pigs, and dogs, the time is only 11 to 25 days.

4. In order to pronounce an animal cured a long period must elapse, since relapses may occur at a very late date (226 days in rats infected with *T. brucei* and treated with atoxyl).

In human trypanosomiasis favorable reports from atoxyl treatment, still continue to come, though the percentage of cures claimed by Koch is probably not reached. Just now good reports are being received from the use of another arsenic compound, introduced by Ehrlich, namely, arseno-phenyl-glycine.

Serum Therapy.—Various normal sera from different animals have been tried with practically no success. A few have prolonged life. Thus Laveran and Mesnil state that human serum injected in sufficient quantities shows manifest action on the disease, and that sometimes cure results in mice and rats. Further, by alternating human serum with arsenic they obtained better results still. Kanthack, Durham, and Blandford showed that animals recovering from trypanosoma infection were immune to further infection. Rabinowitsch and Kempner have made a very careful study of immune serum produced by *T. lewisi*. They have shown that an animal

may be hyperimmunized and that then its serum, in comparatively large doses, inoculated into mice at the same time as the trypanosomes, or twenty-four hours before or after, allows no development of the organisms. Laveran and Mesnil state that the serum causes the rapid destruction of the organisms by the leukocytes, though MacNeal, on the other hand, states that the trypanosomes are destroyed by a cytolytic action of the serum. This immune serum also has a similar action on the trypanosoma of dourine. The serum of animals hyperimmunized against other varieties of trypanosoma is not as active as that obtained by the inoculation of *T. lewisi*.

Koch suggested that an immunity might be established by the inoculation of attenuated parasites, and Novy and MacNeal have succeeded in attenuating cultures of *T. brucei*, and have obtained some success in protecting experimental animals against virulent cultures.

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CHAPTER XLIII.

SPIROCHÆTA AND ALLIES.

The genus *Spirochæta* was introduced by Ehrenberg in 1838, who differentiated it from spirillum by its flexibility. Schaudinn in 1905, thought he saw an undulating membrane in *spirocheta refringens*, so he added this characteristic to the genus and considers that thus its relationship to the flagellated protozoa, genus trypanosoma, is indicated.

Since the appearance of the work of Schaudinn (1905) on the etiology of syphilis, the *Spirochetæ* have been brought into great prominence. Numerous spirochetes and spiral organisms have been described, some associated with *Treponema pallidum* in syphilis, some in other lesions or in the normal secretions of both man and the lower animals; and still the question as to their classification is unsettled. The majority of observers, however, are willing to admit that the structure of many of the varieties classed with this group is more complicated than that of bacteria and that hence the group may be an intermediate one between protozoa and bacteria. For this reason it is still retained in the section on protozoa.

The chief reasons given for considering them protozoa are: (1) their flexibility and the indications in many of longitudinal division and of undulating membrane; (2) the demonstration of forms intermediate between the trypanosomes and the spirochetes (*Sp. balbianii*); (3) the spirochetal forms of certain trypanosomes (*Tr. noctuæ*). In favor of the bacterial nature of spirochetes are: (1) the rigidity of some forms, the lack of undulating membrane in most and of definite nuclear apparatus in all, and the evidence of transverse division in all and of flagella arising from the periplast in some; (2) the cultivation of certain forms (*e. g.*, *Sp. refringens*, by Levaditi; *Sp. Obermeieri*, by Novy) for many generations without development of trypanosome forms.

So far the studies on this group show that the spirochetes and allies probably occupy a position intermediate between the protozoa and the bacteria. We study them here because of the claims that they are closely related to the trypanosomes.

It may be well to note briefly the chief characteristics of the more familiar non-pathogenic ones in order better to understand the relationships between them and the *Treponemo pallidum* and other pathogenic forms.

Material and Methods for Study.—The large *Spirocheta balbianii* is found in the stomach of oysters fresh from salt water. Smaller spirochetes are frequently found in human mouths. When fresh syphilitic or relapsing fever material can be obtained this should be examined. The *Treponema pallidum* (the spiral organism of syphilis), because of its low refractive index, is seen when alive with difficulty by the ordinary microscope, but with the dark-

stage illumination, especially if a drop of distilled water is added to the serum containing the organisms, it is seen distinctly and its motion and structure may be more easily studied. The fluid containing the organisms should be dropped on an ordinary glass slide, covered with a thin cover-glass and well sealed with vaselin as most spirochetes are anaërobic. Material may be obtained from syphilitic lesions as follows: The lesion is first thoroughly washed and dried with distilled water and sterile gauze. Part of the base and margin is then scraped with a curette until the superficial tissue is removed and blood appears. The blood is wiped away with sterile gauze until clear serum begins to ooze. A drop of this serum is used for examination.

Smears should be made as thin as possible and may be stained (1) by GIEMSA according to the method on page 624 (Tr. pallidum stains reddish. See pl. II, Fig. 6); a modification of Giemsa, used by Schereschewsky (see bibliography) has been highly recommended by various workers; (2) by GOLDHORN'S METHOD as follows:

Dye. Water, 200 cm. Lithium carbonate, 2 grams. Methylene blue, 2 grams. (Merck's medicinal or a similar preparation.) This mixture is heated in a rice boiler with a moderate amount of heat until a rich polychrome has formed. This is determined by examining a sample against artificial light and noting the appearance of a distinctly red color. The solution is allowed to cool and the residue is removed by filtering through cotton. To one-half of this filtrate 5 per cent. acetic acid is gradually added until a strip of litmus-paper shows above the line of discoloration a distinct acid reaction. The remaining half of the dye is now added, so as to carry the reaction back to a low degree of alkalinity. A one-half per cent. French eosin solution is now added gradually, while the mixture is being stirred until a filtered sample shows a pale bluish color with slight fluorescence. The mixture is allowed to stand for one day and filtered. The precipitate is collected on a double filter-paper and dried at a temperature not exceeding 40° C. It is then removed from the filter-paper and dissolved in commercial wood alcohol. It is allowed to stand for one day in an open vessel and then filtered.

To use the stain on smears sufficient dye to cover the smear is dropped on an unfixed preparation and allowed to remain for three or four seconds; the excess is then poured off. The slide is now introduced slowly into clean water with the film side down, is held there for four or five seconds and is then shaken in the water to wash off the excess of dye. It is then allowed to dry and is ready for examination. The pallidum stains violet.

Until recently the demonstration in smears of the syphilis spirochete by (3) the *silver impregnation method*, so successfully used by Levaditi in sections has been unsatisfactory. STERN, however, and FLEXNER corroborating him, have gotten beautiful results by the following simple method:

1. Air-dried into 37° incubator for some hours.
2. Ten per cent. aqueous silver nitrate for some hours (Flexner thinks three to four days' exposure better) in diffuse daylight.
3. When the brownish co'or reaches a certain tone (easily recognized after experience) and when a metallic sheen develops the slide is washed well in water, dried and mounted.

The blood cells are well preserved, they have a delicate dark brown contour, and contain fine light brown granules. The spirochetes are deep black on a pale brown and in places a colorless background.

Other spirochetal organisms may be silvered by this method, but as they may be differentiated with greater difficulty than with Giemsa's stain, the latter should always be used as well.

(4) These organisms may also be demonstrated by the India ink method (see p. 47).

The flagella are brought out by Loeffler's method or by the stain recommended by Goldhorn.

Sections are prepared by the SILVER IMPREGNATION METHOD of LEVADITI as follows: Fix small pieces of tissue one-half mm. in thickness for twenty-four to forty-eight hours in formalin, 10 per cent. Wash in 95 per cent. alcohol twelve to sixteen hours. Wash in distilled water till the pieces sink. Impregnate two to three hours at room temperature and four to six hours at 50° C. in the following fluid: Nitrate of silver, 1; pyridine, 10 (added just before using); aq. dist., 100. Wash rapidly in 10 per cent. pyridine. Reduce the silver by placing in the following mixture for several hours: Pyrogallic acid, 4; acetone, 10 (added just before using); pyridine, 15; aq. dist., 100. Harden in alcohol; xylol; paraffin. Levaditi's first method is longer but more reliable. Fix small pieces in formalin, 10 per cent. Harden in 95 per cent. alcohol. Wash in distilled water several minutes. Impregnate three to five days at 37° C. in 1.5 per cent. solution silver nitrate. Reduce twenty-four hours in: Pyrogallic acid, 4; formalin, 5; water, 100. Imbed in paraffin. By these methods the spirochetes appear densely black.

Cultures.—Pure cultures have been obtained of the *Spirocheta dentium* in the following manner: Poured serum agar plates are made of various dilutions of material from the mouth containing these spirochetes. After being kept in the thermostat at 37° C. under anaërobic conditions for nine to twelve days the spirochetal colonies are finished and planted in agar tubes as stick cultures.

Pure cultures of *Spirocheta Obermeieri* by Novy and of *Spirocheta refringens* by Levaditi have been obtained by growing in collodion sacs. (For other culture experiments see below.)

Spirocheta balbianii, Certes (Plate II, Fig. 2).—This great form, next largest known to the *Spirocheta plicatilis* Ehrenberg, may be found in the stomach of the oyster. It is important because it is apparently a transitional form. In fact, it is considered a trypanosome by Perrin and others. Muhlens gives its characteristics as follows: Length 26μ to 120μ, width $\frac{1}{2}\mu$ to 3μ. The body is flattened and possesses an undulating membrane which is visible during life on some individuals. It has 4 to 8 flat, wide spiral coils. Its movements are lively, similar to those of trypanosomes, but more corkscrew-like. During motion its form is apparently easily changed. The rim of the undulating membrane does not end in a free flagellum, but one end of it seems to be attached to a triangular mass of chromatin (basal granule, blepharoplast?) which is a part of the central chromatin material. The nuclear material is arranged in a more or less spiral line along the entire center of the organism.

Before division this nuclear line, after passing through chromasome-like changes, breaks up into pairs, and division takes place longitudinally between them. Division is often incomplete for a time, the two ends remaining attached.

Spirocheta balanitidis.—This is a spirochete found by Simon in *Balanitis circinata* and regarded by some as the specific cause of this disease. Hoffmann and Prowazek describe it as a rather strongly refractive, actively motile band-shaped organism, shorter and thicker than *Spirocheta pallida*, with 6 to 10 coils, staining bluish-red with Giemsa's method and exhibiting an undulating membrane and at either end a periplastic ciliam.

Muhlens thinks this may be identical with *Spirocheta refringens*.

Levaditi has recently reported cultivating it (see below under Treponema pallidium).

The Mouth Spirochetes.—Three varieties of non-pathogenic forms are commonly found in normal mouths.

1. **Spirocheta buccalis**, Cohn (Plate II, Fig. 3*a*).—Length, 10μ to 20μ; thickness, $\frac{1}{3}\mu$ to $\frac{2}{3}\mu$. It has 3 to 10 irregular flat coils. No true cilia have been demonstrated, but Schaudinn, Hoffman, and Prowazek say it has an undulating membrane. It stains violet with Giemsa.

2. **Spirocheta dentium**, Koch (Plate II, Fig. 3*c*).—This is much smaller than the previous form. It is as thin as the pallidum and is somewhat similar to it in refraction, staining qualities, and in the fixity of its coils during motion. It is somewhat smaller and stains a little more easily with Löffler's flagella stain, and flagella have been demonstrated. Neither definite undulating membrane nor nuclear material have been seen. It is 4μ to 12μ long, and has 4 to 20 regular spirals of about the same appearance as those of the *pallidum*. Pure cultures have been made from this spirochete as described above.

3. **A Middle Form** (Plate II, Fig. 3*b*) between these two has been found in the mouth. This also is somewhat similar to the pallidum, but it is larger and has less regular spirals; moreover, it stains more intensely with the blue of Giemsa, only in poorly prepared specimens does it appear red.

Spirocheta refringens (Fig. 177) is also found in the mouth, but it is especially interesting from the fact that it is so often found associated with the *Treponema pallidum* in the various lesions of syphilis. It is not in such large numbers as the *pallidum* and probably bears the relation of a restricted secondary invader. It is generally longer than the pallidum (10μ to 30μ) and much thicker ($\frac{1}{2}\mu$ to $\frac{3}{4}\mu$). In life it is much more refractive. It has 3 to 15 irregular wide, flat spirals which change their shape during motion. Its movements are much more lively than those of *pallidum*. With Giemsa it stains quickly and easily, a blue to a blue-violet tone, according to the length of staining. Schaudinn states that it possesses an undulating membrane. Levaditi claims to have demonstrated terminal cilia for this organism and to have cultivated it in collodion sacs in the rabbit's peritoneum.

Spirocheta Vincenti (Plate II, Fig. 5).—Accompanying the fusiform bacilli in Vincent's angina (see p. 230) are many spirochetes similar to the "middle form" found in the mouth. Whether they are identical with these spirochetes or whether they are a special variety (or, as some think, a second form of the fusiform bacillus) still remains to be determined. Their relationship to the disease is also uncertain.

Miscellaneous Spirochetæ.—Besides the spirochetes found in syphilis, in framboesia, in certain tumors of mice and human beings, and the spiral organisms causing African and European relapsing fevers, all of which will be described below, spirochetes have been found (1) in the normal intestinal tract of mosquitoes and human

beings as well as in the diarrhœal stools of the latter; (2) in the blood of mice, fowls (*Sp. gallinarum*, causing relapsing fever in fowls), and geese; (3) in various ulcerative and gangrenous processes of man. Most of these have been very little studied.

TREPONEMA PALLIDUM (**Spirocheta pallida**).—This organism is found in large numbers in *syphilis*, an infectious disease of human beings, characterized by its long course and by the definite stages of its clinical manifestations.

Historical Note.—Notwithstanding the fact that syphilis is one of the oldest diseases known and studied, only recently has definite light been thrown upon its cause in the discovery of the *Treponema pallidum* (Schaudinn).

Before this it was thought that the bacillus described by Lustgarten and others as occurring in small numbers in the lesions of syphilis bore an etiologic relationship to the disease, but there were no evidences to support this view. Many other bacteria have been erroneously regarded as the probable cause of syphilis.

From time to time various observers have described protozoan-like bodies in syphilitic lesions, but their observations have not been confirmed.

Schaudinn announced early in 1905 that working with Hoffman he found in the fresh exudates of chancre a spiral organism possessing characteristics similar to those of the spirochetes and he named it *Spirocheta pallida*. Later he concluded that this organism was individual enough (that is, it showed no undulating membrane, but possessed a flagellum) to be placed in a separate genus, so he called it *Treponema pallidum*. He thought that the organism was the cause of the disease. Since then there have been extensive studies on human syphilis and on experimental syphilis in lower animals with the result that the work of Schaudinn and Hoffman has been abundantly corroborated and many new facts have been brought out.

FIG. 177

The two spirochetes in the centre are *Tr. pallidum;* the three others, *Sp. refringens.* (Schaudinn and Hoffmann.)

The Organism (Fig. 177 and Plate II, Figs. 6 and 7).—The *Treponema pallidum* is a very delicate structure closely resembling in morphology and staining reactions the *Spirocheta dentium*. It is somewhat longer, 4μ to 20μ long (average 10μ), and thinner, $\frac{1}{4}\mu$ to $\frac{1}{2}\mu$ in diameter. It has four to twenty sharp, deep spirals. The relationship between the length and the depth of the spirals is different in the two species; in *Treponema pallidum* length is to depth as 1 is to 1–1.5 (1μ long and 1μ to 1.5μ deep), while in *Spirocheta dentium* the average relationship is 1:0.5, the spirals being more shallow. The angle of the spiral turn is very sharp in both forms (more than 90°).

Flagella-like anterior and posterior prolongations are often seen in the *pallidum*. The double flagella occurring rarely at one end are interpreted by Schaudinn as beginning longitudinal division. Schaudinn states that the division occurs very quickly (hence the reason why so few dividing forms are seen in stained preparations) and that

it may be followed only by the most experienced observers during life. In the living condition the organism is not very refractive, so it is seen at first with difficulty. Its characteristic movements are rotation on its long axis, quivering movements up and down the spiral which is comparatively rigid, slight forward and backward motion and bending of the entire body. By the use of the ultramicroscope the motility of the organism is clearly seen (Fig. 178).

It stains red as does *Spirocheta dentium* by Giemsa's method, while most other spirochetes stain blue in properly prepared specimens.

Cultivation.—Up to 1909 numerous attempts had been made to cultivate this organism in artificial media, without success. In May, 1909, Schereschewsky reported that he had obtained a culture of a

FIG. 178

Treponema pallidum appearing as bright refractive body on a dark field as shown by India ink or ultramicroscope.

spirochete from syphilitic lesions and blood in the following culture medium: horse serum sterilized by heat (58° to 60° C. [?] until it is of jelly-like consistency, and afterward autolysed at 37° for three days. A piece of tissue excised from the lesion (*e. g.*, base of a papule or part of a lymph node) is inoculated into this medium, and grown at 37° C. The culture begins in three days, but the optimum is reached in 5 to 12 days. Such a culture is always impure and, moreover, it has so far given negative results with specific serum and on animal inoculation.

Mühlens reported in July, 1909, that he had also obtained a culture of a pallidum-like spirochete from syphilitic lymph nodes, grown at first in Schereschewsky's medium and afterward transplanted to broth and grown anaërobically. Animal experiments are being made.

Levaditi and Stanesco about the same time reported growing two species of spirochetes from a case of balanitis. One, a new one, which they found very like pallidum, but nonpathogenic for monkeys; and

which they named *Sp. gracilis;* the other *Sp. balanitidis.* They employed as media (1) collodion sacs in tubes of fluid horse serum; (2) horse or human serum heated to 75° C. These spirochetes were never obtained in pure culture, but in "pure-mixed" cultures, similar to those required by amebas (see p. 536).

Pathogenesis.—So far as is known, syphilis in nature appears only in man. Since 1879, when Klebs stated that he had produced syphilis in monkeys by the inoculation of human virus, various experimenters have reported its transmissibility to these animals by direct inoculation. Most of the earlier reports did not state the exact identity of the animals employed nor did they give details of methods and results.

Metchnikoff and Roux in 1903 produced a typical chancre on the genital mucosa of the young chimpanzee twenty-six days after inoculation. The essential lesion was followed by inguinal adenitis, and thirty days later by a generalized papular eruption. The virus was transferred in this case to lower monkeys. Most monkeys developed a primary lesion only, but some had abundant secondaries.

Since the discovery of the *Tr. pallidum,* experiments on monkeys have been more numerous and have been followed by more helpful results. More has been learned about the course of the infection in man, the evidence in favor of the *Tr. pallidum* being the cause of the disease has been strengthened, and many interesting investigations in regard to immunity have been made.

Important features in regard to course of the infection have been summarized by Ewing as follows: "If the virus is applied to the broken epithelium, a chancre develops, but if similar virus is inoculated into the subcutaneous tissue an initial lesion does not follow, immunity does not develop, and the animals remain susceptible to subsequent inoculation of the epithelium. Yet in several instances Neisser was unable to produce chancres in monkeys which had previously received subcutaneous injections of syphilitic material, indicating that immunity may sometimes appear after such subcutaneous injections. Possibly the leukocytes of the subcutaneous tissue destroy the virus before it can begin to multiply. Hence, small superficial wounds may be more dangerous in man than deep ones. Nevertheless, it is recorded by Jullien that two French surgeons accidentally inoculated by deep needle punctures developed pronounced signs of constitutional syphilis, as attested by Fournier, but failed at any time to show signs of a chancre at the point of inoculation. It remains to be seen whether the observations of the clinicians or those of the experimental pathologists represent the true laws of infection in syphilis.

"In monkeys the virus exhibits a certain choice of epithelium for its entry. The abdominal skin resists the entry, the eyebrows and genitals are most readily inoculable in apes, and the palpebral borders in catharinians. The period of incubation varies from thirty days, on the average, in the chimpanzee, to twenty-three days in lower monkeys, but the shorter the incubation, the shorter and less severe the subsequent disease.

"That the virus circulates in the blood in certain stages of syphilis has been clearly shown experimentally. Although Neisser inoculated human subjects with the blood of florid syphilis without effect, a result which is now explicable, Hoffmann, in two of four experiments, produced syphilis in monkey (*Macacus rhesus*) by inoculating the skin with human blood drawn forty days and six months after the appearance of the chancre. The resulting primary lesions were typical, appearing after the usual incubation and showing a characteristic histological structure and the presence of *Tr. pallidum.*

"Syphilographers are agreed that tertiary lesions are not contagious. Experimental studies have shown, however, that some tertiary lesions are capable of transmitting the disease. Salmon had negative results with an ulcerated gumma in the eighth year of the disease. Yet Neisser produced chancres and secondaries in a gibbon and in a macacus with the material from a non-ulcerated gumma (duration unknown), but the periods of incubation were very long, fifty-one and sixty-eight days. All tertiary lesions do not seem to contain the virus, as Neisser found the material from tuberoserpigenous lesions non-infectious. It appears also that secondary infection and ulceration of tertiary lesions reduces their infectivity. None of these observations invalidates the clinical experience that tertiary lesions are practically harmless for the patient's neighbors, but they suggest greater caution in dealing with tertiary lesions.

"According to Colles' law, a mother who gives birth to a syphilitic infant may not herself contract the disease, but thereafter remains immune to inoculation. This law may be explained by the infection by the embryo or ovum, and the transference of immunity to the mother by the blood or by some other method. The probable mode of origin of the maternal immunity is suggested by an observation of Buschke and Fischer who found spirochætes in the inguinal lymph nodes of such a case which remained entirely free from the symptoms of the disease. The observation, taken with the failure of subcutaneous and intraperitoneal inoculation to infect monkeys, may explain the workings of Colles' law. Levaditi and Sauvage claim to have shown that *Tr. pallidum* is capable of invading the ovum. Finger and Landsteiner found the semen in one case of secondary lues infectious for apes, but in other cases their results were negative. It is, therefore, only necessary to suppose an occasional escape from the genital tract in order to complete the necessary conditions for the infection of the embryo with immunity in the mother.

"Neisser endeavored to determine the degree and duration of the infectivity of the organs of monkeys and found that the virus persists especially in the blood-forming organs, spleen, lymph nodes, and marrow, while in the testicle also the virus is long preserved in active form. The other organs gave entirely negative results."

Syphilis in the Rabbit.—Bertarelli and others have inoculated syphilitic virus into the cornea and anterior chamber of rabbits' eyes and they have obtained ulceration and increased numbers of spirochetes. After several generations of passage, Bertarelli successfully inoculated a monkey from such a cornea.

Syphilis in Man.—The course of the disease is divided into three stages, primary, secondary, and tertiary. The general character of the lesions in these stages is a more or less circumscribed formation of new tissue which is largely made up of small spheroidal cells alone or accompanied by fewer polyhedral cells, and occasional giant cells.

The initial or primary lesion occurs in the form of a papule which develops into the so-called chancre, an ulcer with hardened base. Following this there is hyperplasia of the nearest lymph nodes. These lesions subside and six or seven weeks later the secondary lesions appear in various general eruptions on skin and mucous membranes and in other constitutional disturbances. The tertiary lesions which consist principally of the masses of new tissue called gummata are found throughout the viscera and in the periosteum.

Schaudinn's spirochetes have been demonstrated in practically all the lesions of syphilis (they are most easily demonstrated in the primary and secondary lesions), including the congenital types, in such

numbers and position as to make the majority of workers in this field look upon them as the almost certain cause of syphilis.

The technic first used failed to bring them out as well as that later employed. But the method most recommended, that of silver impregnation, is the one most assailed by the opponents to the view of the organismal nature of the spiral bodies.

The adverse criticism made by Saling, Schultze, and a few others is based upon the fact that silver nitrate impregnates nerve endings and elastic fibrils so that both appear as spiral organisms; even the Giemsa and other stains used, they claim, may stain fibrils of certain tissue in such a way that they look like spirochetes. This criticism has been weakened very lately by the fact that the *pallidum* may be brought out in smears by the silver impregnation method as well as by Giemsa and in section by Giemsa's stain as well as by the silver impregnation (see technique). The use of the ultramicroscope has made certain the fact of the *Treponema* being a living organism.

Immunity.—Natural immunity in syphilis is very peculiar. After the development of the primary lesions, man is usually insusceptible to reinoculation during the active stage of the disease, but during all stages both man and monkey can, in some cases, be reinoculated. Reinoculation in the tertiary stage gives precocious lesions of the tertiary type, gummata and tubercles. Neisser found reinoculation from twenty-four to one hundred and four days after primary inoculation in monkeys sometimes effective, more often negative. During the stage when the skin is refractory to inoculation secondaries develop, showing that there is no complete immunity of the skin to the virus, since the *Treponema* is abundantly present in the lesions. Neisser suggests that cutaneous secondaries develop at periods of relative deficiency of immunity. He has shown that failure to reinoculation is not due to immunity to foreign infection and susceptibility to auto-infection, since the patient's own virus in both man and monkey is ineffective.

Attenuated Virus.—Efforts to secure an attenuated virus to be used for inoculation have been unsuccessful. Fresh material loses its virulence in six hours, and the results of inoculation with such virus in all types of monkeys have been entirely negative. Passage through monkeys does not attenuate the virus, and the absence of secondaries in lower monkeys is apparently no indication of a change in the quality of the virus, but only in the reaction in the host.

Passive Immunization.—Injection of large quantities of serum of syphilitics into chimpanzees has failed to produce definite immunity, although some of Neisser's animals after such treatment failed to take syphilis. The serum of a monkey cured of syphilis and subsequently injected over a period of fifteen months with the blood of syphilitic subject in roseolar stage was without therapeutic effect. However, this serum dried and powdered prevented the chancre when placed on the site of inoculation one hour after the virus.

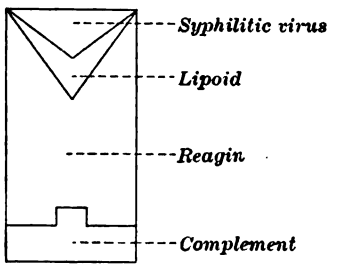
The Wassermann Reaction.—Wassermann, Neisser, and Bruck were the first to apply the Bordet-Gengou phenomenon (see Part I) to the

diagnosis of syphilis. According to many workers, enough work has been done since then to establish its value as a diagnostic test, but a few still think it not dependable. Some interesting points have been brought out in connection with this study. In the first place, it has been shown that the antigen helping to bring about the reaction is not specific since alcoholic extracts from normal organs (liver, kidney) react as well as those from syphilitic organs. With further study it has been found that certain lipoids (lecithin) possess great antigenic power, though not quite as great as the total extracts. Swift (see bibliography) represents the "luetic system" as shown in Fig. 179.

The Explanation and Technique of the Wassermann Test for Syphilis.—Five components are present in this test: Antigen, antibody, complement, hemolytic amboceptor, and blood cells.

As originally employed the antigen consisted of a watery extract of the liver of a syphilitic fœtus, and this is still preferred by some workers, but extracts of normal liver, either watery or alcoholic, and also of many other organs, especially guinea-pig hearts, are considered by most authorities to be equally effective. For the watery extracts the finely divided tissues are mixed with normal salt solution in the proportion of 1 to 4, agitated at room temperature for twenty-four hours, centrifuged and the supernatant fluid drawn off into sterile vessels and kept in the ice-box till needed. Alcoholic extracts are also made from the fresh minced or crushed tissue in 96 per cent. alcohol.

FIG. 179



The antibody is contained in the patient's serum; the blood being drawn from a vein, is allowed to coagulate or is centrifugalized, and the serum is pipetted off and inactivated by heating in a water-bath for one-half hour at 56°.

The complement used is that in fresh guinea-pig's serum.

The hemolytic amboceptor is obtained by inoculating an animal with the washed blood cells of another species (as a rule, rabbits inoculated with sheep cells). The blood cells, after washing four to five times in salt solution to free them from serum, are suspended in fresh salt solution and inoculated either subcutaneously or intraperitoneally at intervals of five to ten days for from four to five injections; and nine to ten days after the last inoculation the blood of the rabbit is drawn, centrifugalized, the serum pipetted off, inactivated at 50 degrees for one-half hour and stored in the ice-box.

The blood cells used in the test (generally those of sheep), after being freed from serum by repeated washing in salt solution, are suspended in fresh salt solution in proportion of 5 per cent.

Antiseptic precautions to avoid bacterial contamination are used

throughout, and all glassware must be thoroughly cleansed and made neutral in reaction.

The amount of hemolytic amboceptor necessary to dissolve the blood cells, and the optimum proportion of antigen and antibody to fix complement must be determined by preliminary titrations in each case, after which, as originally described by Wassermann, the materials for the test are each diluted with salt solution in such proportion that 1 c.c. will contain the desired amount, making 5 c.c. in each tube in the completed test, the amboceptor being used in double the hemolytic dose, and the deficiency in the contents of the control tubes, from which one or more factors have been omitted, being supplied by added salt solution. A specimen of normal serum and one of proved syphilitic serum must always be examined at the same time with the serum which is being tested for syphilis, and in addition to this a full series of control tubes must be used in each case.

In making the test the antigen antibody and complement are measured into the tubes and these placed in the incubator for one hour at 37 degrees to allow time for the fixation of complement. The hemolytic amboceptor and blood are then added and the tubes returned to the incubator for two hours, after which they are shaken, and the results read after further standing at room temperature or in ice-box.

This test has been very widely used and positive results have been obtained in an immense number of cases. On account of the difficult technique involved it can only be of use in the hands of experienced workers. Positive results have been reported in a number of other diseases as well as syphilis, but in many cases these results have not been generally accepted, and in other cases the diseases showing positive reaction as yaws, leprosy, dourine, etc., have been as a rule confined to the tropical countries, or else the positive reaction has been found only during a limited stage of the disease, as in scarlet fever or the differential diagnosis is otherwise marked, as with tuberculosis. The general opinion at the present time seems to be that while theoretically the test is open to criticism, as not strictly specific, it is, nevertheless of great practical value in the majority of cases of syphilis.

Many investigations of the test have been proposed; but, with the exception of the Noguchi method, these have not found extensive acceptance. In this method human blood cells are used together with antihuman hemolytic amboceptor from a rabbit, thus eliminating the source of error due to the occasional presence of hemolytic amboceptors for sheep blood in human serum. Antigen, antibody and guinea-pig complement are prepared in the form of reagent papers which remain stable if kept perfectly dry, thus avoiding many of the difficulties of the Wassermann method. As to the comparative accuracy of the two methods, opinions are still divided. According to a recent writer by a combination of the two methods correct interpretations were obtained in 98.2 per cent. of 1,400 cases.

The attempted explanation of the nature of the reaction has given rise to much discussion. It has been found that syphilitic serum is

able to cause complement binding not only with organ extracts, but also as noted above with numerous inert and apparently unrelated substances, such as lecithin, cholesterine, vaseline, etc., and it is, therefore, no longer possible to regard the reaction as due to the action of an antigen with its specific amboceptor in the patient's serum, and the term amboceptor is therefore without significance in this connection, although still used as a matter of convenience. Bordet's absorption, therefore, seems to many a rational use, and many observers are inclined toward a simpler chemical explanation of the reaction. On the assumption of some precipitation as the underlying factor it has been proposed to use precipitation tests of various kinds such as those of Fornet, Porges and Meier, Dansner, Sachs, and Altmann and Noguchi in place of the complement binding test, but the latter has been found by most observers to be more accurate than the proposed substitutes. Beyond the evidence as to the liquid character of the active substance furnished by the fact of its solubility in alcohol nothing is definitely known at the present time as to the true nature of the reaction giving rise to complement binding.

Spirochetes in Frambœsia tropica (Yaws).—Castellani in 1906 announced that he had found in yaws a spiral organism which he called *Spirocheta pertenuis*. He determined that monkeys are susceptible to inoculations with material from yaws patients apparently containing only this spirochete. Such material filtered is inert. Monkeys successfully inoculated with yaws do not become immune for syphilis, neither do those having had syphilis become immune for yaws. Further specific characteristics between the two diseases are brought out by means of the Bordet-Gengou reaction. The spirochete, however, is morphologically similar to the *Treponema pallidum*, and should therefore be called *Treponema pertenuis*. His work has been corroborated by several observers.

Spirochetes in Tumors (see Plate II, Figs. 9 and 10).—Loewenthal, Borrel, and others found spirochetes in small numbers in certain mouse tumors. Ewing and Beebe found a few in some dog tumors and others have reported their occasional presence in both ulcerating and non-ulcerating human tumors, but apparently never in sufficient numbers to account for the tissue reaction. Gaylord, however, found that in repeated transplants of a mouse tumor, as the inoculated material became more virulent the number of spirochetes greatly increased. Calkins studied the morphology of Gaylord's spirochete and decided that it is a distinct species. He has also found this species in primary as well as in transplanted tumors. It is much shorter and thicker than the *pallida*, and has blunt ends. It closely resembles the spirochetes found comparatively frequently by Tizzer and others in apparently normal mice, though the possibility of infection in these cases was not ruled out.

Spirocheta Obermeieri (Sp. recurrentis) and Allies.—These organisms are classed with the spirochetes as protozoa by Schaudinn, Hartmann, Mühlens, and others, but by Norris, Novy, and others they

are still placed with the bacteria. Novy and Knapp have made extensive studies of *Sp. Obermeieri*, the cause of relapsing fever in Europe) as well as of *Sp. Duttoni* (the cause of tick fever), spirochetes from American relapsing fever, and *Sp. gallinarum* (fowl spirochete) and considers that he has demonstrated their bacterial nature and that many, if not all, spirochetes should be placed in this group.

Spirocheta Obermeieri was first observed by Obermeier in 1873 in the blood of persons suffering from relapsing fever. It was found in large numbers during the height of the fever, it disappeared about the time of the crisis, and reappeared during the relapses. It was not found in other diseases. Obermeier considered it the cause of the disease, and his views were shown to be correct by the production of the disease in man and ape through experimental inoculation.

FIG. 180

Photograph of *Sp. Obermeieri* showing terminal flagellum. × 3000. (After Novy.)

Morphology.—The organisms are long, slender, flexible, spiral or wavy filaments, with pointed ends, from 16μ to 40μ in length and from one-quarter to one-third the thickness of the cholera spirillum ($\frac{1}{2}\mu$ to $\frac{3}{4}\mu$). They stain somewhat faintly with watery solutions of the basic aniline dyes, better with Loeffler's or Kühne's methylene-blue solutions, or with carbol-fuchsin; best with the Romanowsky method or its modifications. They are negative to Gram. Novy has demonstrated a terminal flagellum (Fig. 178). There are three to twelve wide, irregular spirals.

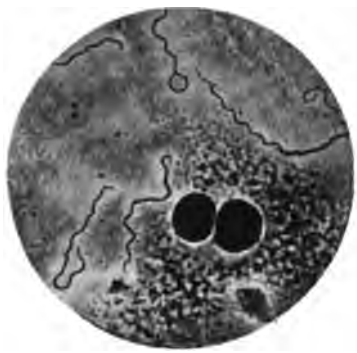
Biologic Characters.—In fresh preparations from the blood the spirochetes exhibit acitve progressive movements, accompanied by very rapid rotation in the long axis of the spiral filaments or by undulating movements. They are found only in the blood or blood organs, never in the secretions, and only during the fever, not in the intermissions, or at most singly at the beginning of, or for a short time after, an attack.

When kept in blood serum, or a 0.6 per cent. solution of sodium chloride, they continue to exhibit active movements for a considerable time. They may be preserved alive and active for many days in sealed tubes. They are killed quickly at 60° C., but they remain alive for some time at 0° C. Unsuccessful efforts to cultivate them in artificial culture media have been made from time to time. Koch has observed an increase in the length of the sprilla and the formation of a tangled mass of filaments. Novy has finally succeeded in cultivating them in celloidin capsules placed in the peritoneum of rats.

Pathogenesis.—In man, whether the disease is acquired naturally or by artificial inoculation, the organism causes the following symptoms:

After a short period of incubation the temperature rises rapidly, remains high for five to seven days, and then returns to normal by crisis. About seven days later there is another sudden rise of temperature, but this time the crisis occurs sooner. A second or third relapse may occur. The organisms increase in numbers rapidly in the blood from the beginning of the fever, large numbers often being found in every microscopic field. They began to disappear a short time before the crisis, and immediately after the crisis it is practically impossible to find them in the circulating blood. The mortality varies in different epidemics from 2 to 10 per cent. When monkeys are inoculated with human blood containing the spirilla, they become sick about three and a half days later, but show only the initial febrile attack or, at the most, an occasional short relapse. The organisms are found to have the same relation to the pyrexial periods as in man. Blood from one animal taken during the fever induces a similar febrile paroxysm when inoculated into another animal.

FIG. 181



Spirocheta Obermeieri blood smear. Fuchsin. × 1000 diam. (From Itzerott and Niemann.)

Metchnikoff showed that during the intermissions when the spirilla disappeared from the circulating blood they accumulated in the spleen and were ingested in large numbers by certain phagocytes and finally were destroyed.

According to Lamb, a certain amount of immunity is conferred upon monkeys (*Macacus radiatus*) soon after an attack, but it disappears quickly. If the serum is removed during this time it is found to have some protective action when mixed with the blood containing spirilla, and also to cause agglutination of the organisms. Novy (1906) showed that a powerful specific germicidal body exists in the blood of rats during and after recovery, notably in the blood of hyperimmunized rats. An immunizing body probably distinct from this is also present. He also showed that passive immunity can be imparted by injections of recovered or hyperimmunized blood, that both active and passive immunity may last for months, and that the serum has both a preventive and a curative action.

Infection probably occurs through the bite of blood-sucking insects.

Spirocheta Duttoni.—The organism shown by Dutton (1905) to be the cause of African tick fever is very similar morphologically to *S. Obermeieri*, but Novy Fränckel, and others have shown slight differences which make them believe that it is another variety, if not another species of this group. Dutton demonstrated that this organism can be transferred to monkeys by the bites of young ticks (*Ornithodorus moubata*) at their first feed after hatching from infected parents. He

accidentally demonstrated the fact that the disease can be inoculated into human beings through a cut surface, for after a wound received at autopsy he developed the disease which eventually caused his death.

Spirocheta Carteri.—This spirochete was described by Carter in 1877 as causing relapsing fever in Bombay. Monkeys were inoculated by Carter successfully with the human blood containing this spirochete

Spirochetes from Relapsing Fever in America.—Recently Darling has reported a study of the relapsing fever of Panama. He isolated the organisms in two cases and studied their characteristics. He finds they agree with those reported by Carlisle, Norris, and Novy for the organisms isolated by Norris, but they can only be differentiated from the other relapsing fever spirochetes by animal inoculations and by the disease in humans. Moreover, he finds that in all probability a polyvalent serum may be necessary for cure, since the serum from one strain did not protect against the other strain.

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CHAPTER XLIV.

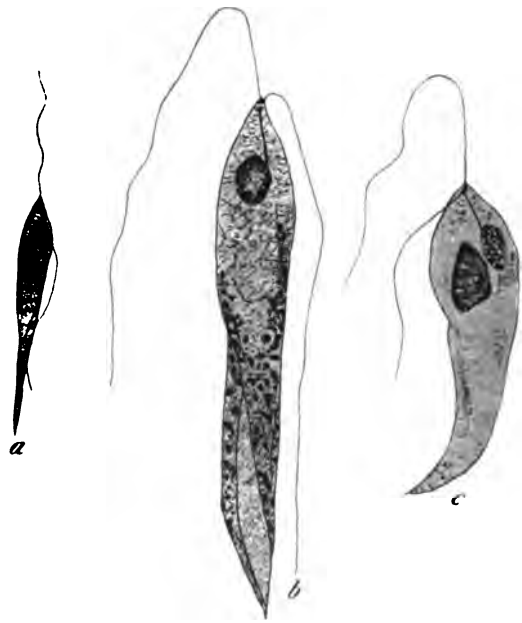
BODO. POLYMASTIGIDA. CILIATA. SPOROZOA.

BODO LACERTÆ (GRASSI).

Bodo lacertæ is frequently found in the intestinal contents of most of the higher animals, hence it is easily obtained for class study. A species of the *Bodo* has been observed in human urine (*Bodo urincarius*), but it is probably a harmless invader.

It is lancet- or wedge-shaped, the posterior part of the body being turned a half to a whole spiral on itself. It possesses two characteristic flagella, equal in thickness but unequal in length. In motion the

FIG. 182



Bodo lacertæ (Grassi). *a* living; *b* and *c* stained; *b* type without, *c* type with chromidium (1000 : 1). (After v. Prowazek, from Kisskalt and Hartmann.)

longer one is directed forward, while the shorter is carried backward, functioning as a rudder, or a towing flagellum. Both flagella spring from basal granules which are well demonstrated by the iron hæmatoxylin stain. They are situated in the extreme anterior part of the body and are attached to the nucleus by a delicate fibril (Fig. 182, *b*, *c*). The movement of the organism is characteristic, it consists in

a rapid irregular swimming in various directions with the anterior flagellum moving from side to side. The body itself shows a slightly sinuous motion.

There are two types of nuclei seen. First, the typical vesicular nucleus most frequently seen among the flagellates. This is round and has a definite membrane about which chromatin is arranged in irregular masses. In the center, or eccentrically placed, is a compact karyosome. Iron hæmatoxylin preparations bring out an achromatic network between the chromatin masses and the karyosome. In the living condition the nucleus appears as a greenish glistening refractive vesicle (Fig. 182, *a* and *b*).

The second type of nuclear apparatus is seen in smaller organisms. This is a similar nucleus except that it is smaller and more compact; posterior to this is another nuclear-like body, varying much in shape and arrangement of chromatin (Fig. 182, *c*). This is the sexual chromidia.

The cytoplasm appears in iron hæmatoxylin stained specimens as finely reticular. It contains many deeply stained granules. There is no mouth opening. Food is taken in by osmosis.

In propagation, the two types just described develop differently. The first or ordinary type forms round division cysts. The flagella disappear and a delicate cyst membrane is formed. The increase in the size of the nucleus and the subsequent division may be followed in life. It lasts about twenty minutes. After a single or, more seldom, a double division of the cell, the daughter cells, while still within the cyst, form their flagella, become very motile, finally break the cyst wall and swim out.

The second type increases, in the free living condition by longitudinal division. The basal granules divide, the principal nucleus divides by mitotic division, the chromidia by amitosis. This all can be seen in hæmatoxylin preparations. Sexual division in this species occurs in cysts by autogamy. It is not easily followed in life because of the high refraction of the cyst. The changes must therefore be studied in specimens stained with iron hæmatoxylin.

They are shortly as follows: The nucleus becomes larger and about its membrane appear small spheres of chromatin which finally leave the nucleus and gather together, forming the so-called chromidial or sexual nucleus, while the original or somatic nucleus gradually degenerates. The new nucleus divides amitotically into two daughter nuclei, from these two smaller parts are then separated, as reduction nuclei, which also degenerate. The remaining parts of the two nuclei increase in size and then fuse to form a new nucleus. The organism may then leave the cyst or the cyst may become a lasting cyst and serve to infect a new host.

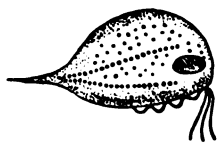
Besides this method of fructification by autogamy in a cyst, is seen, though seldom, a copulation between two individuals of different sizes which afterward become encysted and divide into two to sixteen daughter flagellates.

POLYMASTIGIDA.

The order polymastigida consists of flagellates having several flagella projecting from different parts of the body. The majority of the forms known are parasitic in certain fish.

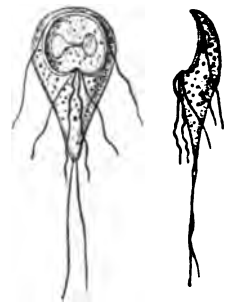
Trichomonas Vaginalis—Donné in 1837 described a form which he found in the human vagina, and which he therefore called *Trichomonas vaginalis*. It has been found by other observers to be a frequent habitant of the vagina at all ages. It has also been found a few times in the acid urine of males.[1] The mode of infection of the female is unknown. The body of the parasite at rest is pear-shaped, but during action its amœboid movements cause it to assume various shapes. The size varies from 12μ to 25μ long and 8μ to 15μ wide. The protoplasm is finely granular, excepting for two rows of larger granules which begin on either side of the nucleus and converge posteriorly. From the anterior part project three to four flagella, which seem to begin at a basal thickening near to, or con-

FIG. 183



Trichomonas vaginalis. (Blochmann.)

FIG. 184



Lamblia intestinalis. (Schewiakoff.)

nected with, the more or less oval, indistinctly vesicular nucleus. From the origin of the flagella an undulating membrane extends backward. The body also seems to possess a certain linear structure connected with the membrane. Contractile vacuoles have not been seen.

Trichomonas hominis Davaine.—This form, found frequently in the human alimentary canal, is very similar to the *Trichomonas vaginalis*, but it is smaller and more pear-shaped. It has been found often in acute diarrhœas, but no causal relation between it and the pathologic process has been shown.

A similar form has been seen a few times in lung gangrene, aspiration pneumonia, and bronchiectasis.

Lamblia intestinalis (Lambl, 1859), a flagellate belonging to this group, parasitic in the small intestines of mice, rats, rabbits, dogs, cats, and sheep, has also been found occasionally in the human in-

[1] It has been found by us in the slightly acid urine of a colored woman, age 45, suffering from acute nephritis. None were found in the vagina in this case.

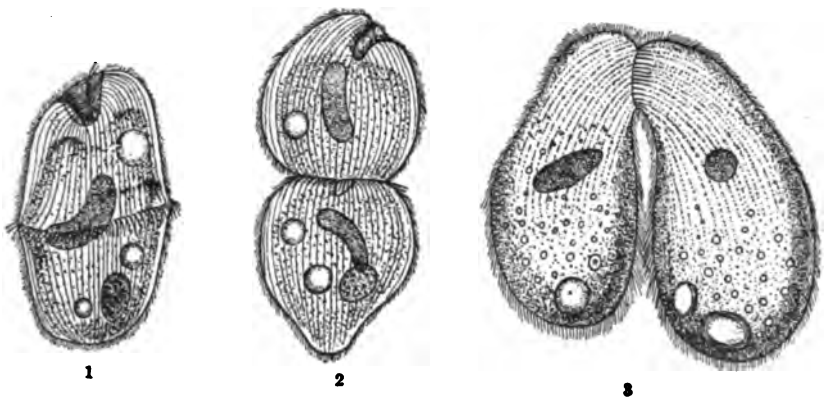
testines. It is beet-shaped, bilaterally symmetrical, 10μ to 21μ long and 5μ to 12μ wide, possessing flagella 9μ to 14μ long. Anteriorly, this species has a characteristic concavity, the rim of which seems to be contractile, forming a sucking apparatus. The eight flagella of the organism are arranged in pairs: one anteriorly, two laterally, and one posteriorly. The nucleus is situated anteriorly and has a central constriction. The protoplasm of the body is thick and hyaline. Contractile vacuoles have not been seen. Schaudinn has recently observed encystment, copulation, and complicated nuclear changes in this organism.

Infection follows the ingestion of the cysts with unclean food. The parasites fasten themselves to the free surfaces of the epithelial cells by their sucking apparatus, but seem to exert no harmful influence on their hosts. They have been found most frequently in poor children who play often in dirt containing the cysts. Repeated small doses of calomel will cause their disappearance from the fæces.

CILIATA.

The *Ciliata* (Fig. 185) belong to the most complex of the protozoa. They possess a definite entoplasm containing nuclei and food vacuoles, and a definite ectoplasm containing basal granules from which arise the cilia which give the group its name. They have

FIG. 185



Balantidium coli: 1, 2, stages of division; 3, conjugation. (After Leuckart.)

organoid structures which receive the food, some have definite mouth openings, indeed, and definite places for excreting waste products. The food vacuoles may contain acid or alkaline digestive products. The nuclear material is differentiated into two forms, a large macronucleus and a much smaller micronucleus. The function of the macronucleus is supposed to be vegetative, and that of the micronucleus reproductive. The macronucleus varies in size and shape and is completely filled with an alveolar chromatin. The micronucleus also varies in size and shape, but unless in reproductive phases

is generally vesicular in structure, with the chromatin heaped in one mass. Division of the nuclei takes place by mitosis in the case of the micronuclei, and by amitosis, as a rule, in the case of the macronuclei. Under conditions unfavorable for growth the ciliata may encyst.

Conjugation is necessary to the life activity of these organisms. The phenomena of conjugation in the ciliata has been well worked out. The micronuclei play the most important part, whereas the macronuclei simply break up and disappear in the protoplasm.

According to the arrangement of the cilia, the ciliata are divided into the four orders given in the general classification. Among these, the second, the order of the Heterotricha, interests us. In the Heterotricha the cilia are uniform over most of the body, while a specialized set fused into a series of firm vibratory plates are found about the mouth.

Only one genus, *Balantidium*, has been observed in man.

Balantidium coli (Malmst, 1857). The body of this infusorium is egg-shaped, with a funnel-shaped mouth opening. The surface of the body is covered with a pellicula, under which is a distinct ectoplasmatic sheath containing rows of basal granules from which the short, fine cilia arise.

The cloudy entoplasm contains fat and starch granules and may contain many red blood cells and other food particles from the host. Two contractile vacuoles have been seen. Posteriorly there is a small prominence marking the place where excreta are expelled. The chromatic macronucleus is bean-shaped, and the vesicular micronucleus is nearly spherical.

Division is transverse, the macronucleus dividing by simple constriction and the micronucleus by mitosis. Conjugation has been observed. Spherical cysts surrounded by a thick membrane are formed.

Balantidium coli has been found in the large intestines of human beings and of swine—probably two distinct varieties. The variety occurring in human beings has been found in about 60 cases, principally in Sweden, but also in Russia, Scandinavia, Finland, China, Italy, Germany, and the United States. Most of these cases were suffering from severe chronic intestinal catarrh, often accompanied by bloody diarrhœa. A number of observers (Strong, Brooks, and others) think the balantidium the primary cause of the catarrh, while others believe it to be a harmless inhabitant of the intestines, or at least only a secondary excitant (Opie, Mahnsten, Doflein, and others).

Schaudinn has described two additional species of balantidium found in the human intestines, which he has called, respectively, *Balantidium minutum* and *Nyctootherus faba*, probably both non-pathogenic.

THE SPOROZOA.

The Sporozoa are a group of exclusively parasitic protozoa of very widespread occurrence, living in the cells, tissues, and cavities of animals

of every class. Generally they are harmless, but some varieties may produce pathologic changes and even fatal diseases severely epidemic.

As their name indicates, they are all characterized by reproduction through spore formation, but they exhibit the utmost diversity of structural and developmental characteristics. As a rule, each species is parasitic on one kind of tissue of a particular species of host. They are generally taken into the system in the spore stage either (1) with the food of the host, (2) by the bites of insects, or (3) by inhalation. The spore membranes are dissolved by the fluids of the host, and thus one or more germs or sporozoites are set free to bore into the special cells of the host. Here they grow, some remaining permanently intracellular, others only in the young stages. The latter either pass different phases of their more or less complicated life history in different parts of the body of one and the same host or they pass some phases of their life cycle in the cells of an intermediate host.

The sporozoa vary widely in size as well as in other characteristics. From the smallest, several of which can be contained in a single blood cell, there are all gradations in size up to those that may be seen by the naked eye (*Porospora gigantea*, 16 mm.).

Besides being characterized by the power to produce more or less resisting spores, the sporozoa are also characterized by the fact that as a class they possess none of the special organs found in other protoza for ingesting or digesting solids. Many develop flagella during sexual phases or show amœboid movement during certain stages of their life cycle, but the flagella and pseudopodia are organs of locomotion and not of nutrition. Food vacuoles or contractile vacuoles have not been found.

The life cycle of a typical sporozoan is represented after Schaudinn in Fig. 186.

A somewhat similar cycle may be followed in the study of the *Coccidium cuniculi* of the rabbit, a description of which is given below. The other forms in this group, which are parasitic in man, or which are of some medical interest, are, besides a number of not fully studied *Coccidia*, *Nosema*, *Sarcocystis*, *Babesia* and *Plasmodium malariæ* and its allies.

COCCIDIUM CUNICULI (RIVOLTA, 1878).

The *Coccidium cuniculi* is a sporozoan parasite of the rabbit. Young rabbits are especially susceptible, and extensive epidemics may occur in breeding houses.

Material and Methods for Study.—Rabbits infected with *Coccidium cuniculi* are often found, and the whole course of the infection may be followed with more or less ease.

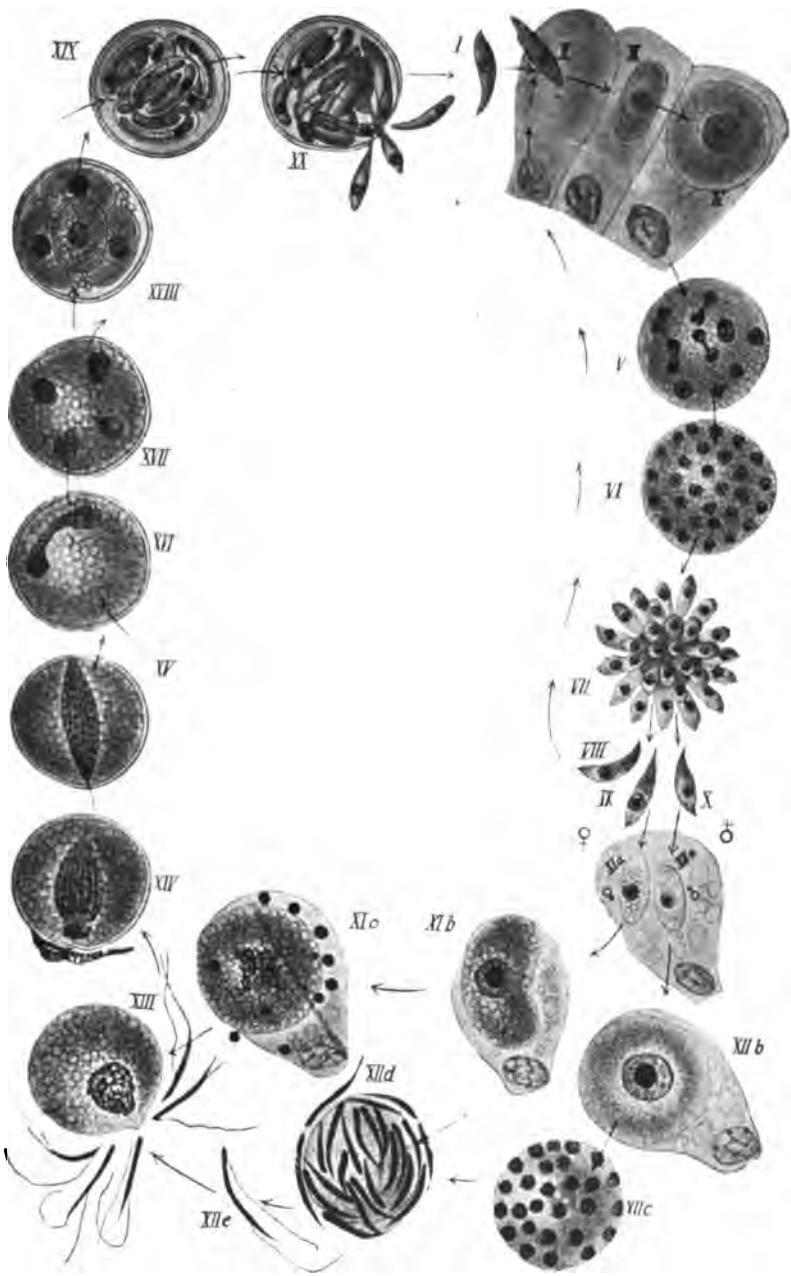
A certain amount of development may be watched in hanging drops of salt solution emulsions. Sections and smears are prepared as described on p. 537.

The cysts are stained with difficulty. It is recommended that a thin solution of Delafield's or Grenacher's hæmatoxylin be used for twenty-four hours followed by eosin. Heidenhain's iron hæmatoxylin stain (p. 537) followed by Bordeaux red is especially good for sections.

DESCRIPTION OF FIG. 186. (After Schaudinn.)

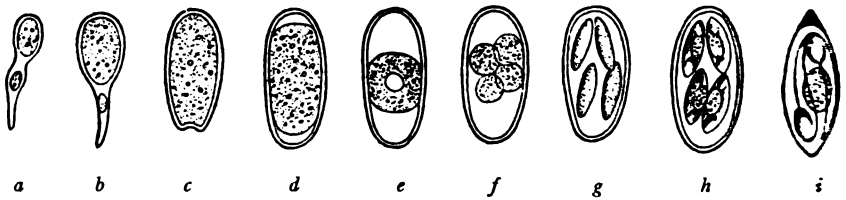
The life cycle of *Eimeria schubergi*. *I* to *VII* represent the asexual reproduction or schizogony, commencing with infection of an epithelial cell by a merozoite or a sporozoite; the merozoite after stage *VII* may start again at stage *II*, as indicated by the arrows, or it may go on to the formation of gametocytes (*IX* to *XII*). *IX* to *XIV* represent the sexual generation, the line of development becoming split into two lines—male ♂ and female ♀—culminating in the highly differentiated gametes, which conjugate and become again a single line, shown in *XIV* and *XV*. The zygote thus formed goes on to the production of spores, *XVI* to *XX*. *I* to *IV* represent epithelial cells showing penetration of a merozoite or a sporozoite and its change into a schizont. *V*, the nucleus of the schizont dividing. *VI*, numerous daughter-nuclei in the schizont. *VII*, segmentation of the schizont into numerous merozoites, about a central mass of residual protoplasm, which in this figure is hidden by the merozoites. *VIII*, merozoite passing to reinfect host cell and repeat the process of schizogony. *IX*, *X*, merozoites to be differentiated into male and female gametocytes. *XIa*, and *XIIa*, the two gametocytes within a host cell; the microgametocyte (♂) has fine granulations; the macrogametocyte (♀) has coarse granulations. *XIb*, an immature female gametocyte within a host cell. *XIc*, a female gametocyte undergoing maturation, still in the host cell. *XIII*, mature macrogamete, freed from the host cell, and sending a cone of reception toward an approaching microgamete. *XIIb*, a full-grown microgametocyte within a host cell. In *XIIc* the nucleus of the microgametocyte has divided up to form a great number of daughter-nuclei. In *XIId* the nuclei of the last stage have become microgametes, each with two flagella. *XIIe*, represents the free microgametes, swimming to find a macrogamete. *XIV*, the zygote (fertilised macrogamete), surrounded by a tough membrane or oöcyst, which allows no more microgametes to enter, and containing the female chromatin, which is taking the form of a spindle, and the male chromatin in a compact lump. *XV*, the chromatin from these two sources united and no longer distinguishable as male and female. *XVI*, the nucleus of the zygote dividing. In *XVII* four daughter-nuclei are formed—the nuclei of the sporoblasts. In *XVIII* the four sporoblasts become distinct, leaving a small quantity of residual protoplasm; each sporoblast has formed a membrane, the sporocyst. In *XIX* within each sporocyst two sporozoites have been formed about a sporal residuum. In *XX*, the sporozoites, becoming free by bursting the sporocysts, pass out through an aperture, in the wall of the oöcyst, and are ready to enter the epithelial cells of the host. (From Lang.)

Fig. 186


XIX
XI
I
XIIII
XVII
VII
V
XIV
XIII
XIIe
XIId
XIIc
XIb
XIa
VIIc
XIIb
V
VI
VII
VIII
II
X
♀
♂

The symptoms of the disease are fever, diarrhœa, yellowish mucous discharge from the nose and mouth, and progressive wasting. The liver is much enlarged and shows throughout its substance variously sized gray-white tubercles, generally surrounded by a capsule, and containing a slimy mass of degenerated host cells, in which are embedded the parasites. The parasites are also found in the fæces and in the epithelial cells of the intestines, gall-ducts, and liver. The acute stage of the disease lasts about three weeks. The contents of the coccidial tumors in animals that have withstood the infection may later be emptied, leaving only a mass of cicatricial tissue. In such animals

FIG. 187



Showing spore formation in *Coccidium cuniculi* from the liver of the rabbit: *a* and *b*, young stage in the epithelial cells of the gall-ducts (the small oval is the cell nucleus); *c*, *d*, and *e*, the fertilized oöcyst; in *d* the protoplasm is beginning to shrink away from the cyst wall, and in *e* it has contracted into a spherical form; *f*, segmentation into four sporoblasts; *g*, Elongation of the sporoblasts to form spores; *h*, four complete spores in the oöcyst; *i*, single spore more highly magnified, showing the two sporozoites and a small quantity of residual protoplasm. The life cycle has been fully worked out by Simon. (After Balbiani, from Doflein.)

the oöcysts may remain for a long time in the gall-bladder and intestines, and by passing out gradually with the fæces may provide a source of infection for other animals. The infection is carried by food soiled with cyst-containing fæces. The cysts pass with the food into the stomach, where the cyst wall and the spore sac are destroyed and the sporozoites are set free. The motile sporozoites pass through the ductus choledochus into the liver, some probably passing into the intestines and infecting the cells directly, a later infection of the intestines occurring from forms developed in the liver. The organism develops within the epithelial cells of the liver and gall-ducts until the cells are finally broken down and tissue cysts are formed, within which, after more or less complicated changes, cysts of the parasite are again formed.

A few cases of human infection of the liver with the *Coccidium cuniculi* have been reported. The *Coccidium hominis* Rivolta, found a few times in the human intestines, as well as similar coccidia, found in the intestines of lower animals, may belong to the same species.

Coccidium bigeminium (Stiles) is found in the fæces of dogs, cats, polecats, and possibly human beings. The organism is characterized by the division of the oöcyst into two united cysts, containing four spores. The size is 8μ to 15μ. The life cycle is not well known.

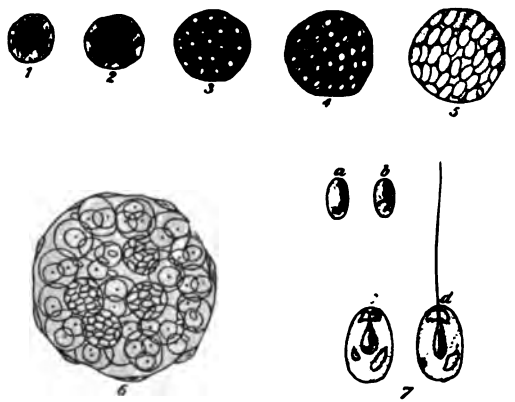
Rhinosporidium kinealyi is the name given by Minchin and Fantham (1905) to a probable sporozoan found in the nasal mucous

membrane of certain cases from India that were troubled with hemorrhagic nasal polyps. Nais reported four similar cases and Beattie another in 1906.

MYXOSPORIDIA.

The *Myxosporidia* belong to one of the most populous and abundant groups of the sporozoa, showing great structural variation as well as divergence in mode of life. Nevertheless the members have, as a group, the following well-marked characteristics: The trophozoite is ameboid; spore formation begins at an early period and proceeds continuously during the growth of the trophozoite; the spores are pro-

FIG. 188



Nosema bombycis; 1 to 5, spore formation; 6, infected follicle of testicle; 7, spores; *a*, *b*, fresh; *c*, *d*, treated with nitric acid. The acid causes them to swell up and increase in size by at least a half, at the same time making the polar capsule distinct. In *d* the filament is extruded. (After Balbiani.)

duced endogenously—*i. e.*, within the protoplasm of the trophozoite, and each spore always possesses one or more very distinctive structures, "the polar capsules" (Fig 188, *c*, *d*).

The myxosporidia are habitants of fishes, reptiles, arthropods, and some other classes of animals. They infest especially arthropods, causing often most virulent epidemics. The most interesting member of this group is *Nosema bombycis*, the cause of silkworm disease (Pébrine). The organism forms many small spores each with one polar capsule. The spores which are carried by the food into the intestinal canal of the caterpillar, pass through the walls of the intestines, and infect all organs. Spores found in the ovary may be carried over to the newly hatched silkworms, thus causing a further dissemination of the disease.

The other member of this group, of interest here, is *Nosema lophii* Doflein. Its interest lies in the fact that it has been found to infect only the ganglion cells of the sea-devil, thus apparently resembling in its parasitic nature the organism causing hydrophobia.

SARCOSPORIDIA[1]

This order is very little known but, considering the fact that through eating uncooked infected meat, it may be found in man, though rarely, its chief characteristics should be noted here.

The *Sarcosporidia* are parasites of the striped muscles or connective tissue of some of the warm-blooded vertebrates (various birds and mammals). They are found in the adult state in elongated sacs known as "Rainey's" or "Miescher's Tubes." (Fig. 189.)

The trophozoite is a motionless elongated body, limited by a cuticle growing into a complicated structure. Spore-formation begins at an early stage and proceeds during the growth of the trophozoite (*Neosporidia*) which may become very large. The spores, which are many, are minute sickle-shaped

FIG. 189

FIG. 190

Sarcocystis tenella in a muscle cell of a sheep, from the striped muscle of a swine. A full grown cyst, showing radiately striped membrane, which is broken on right side. Bertram.

Sarcocystis miescheri. *a*, small cells from a cell group. *b*, loosening of the protoplasm from the cell wall. *c*, *d*, sickle-shaped bodies (sporozoites) formed from the small cells. (From Wasielewski.) Manz.

or spindle-shaped mononucleate bodies with a delicate envelope and at one pole an oval striated body which represents the polar capsule found in the myxosporidia. (Fig. 190.)

In some cases the cyst wall calcifies and the contents of the cyst degenerate, with apparently no harm to the host; in other cases the cysts burst and their contents spread into the surrounding tissue, producing abscesses and tumors as with many myxosporidia and sometimes causing the death of the host.

The symptoms of sarcosporidiosis in the pig are paralysis of the hind extremities, a skin eruption, and general systemic symptoms, as increased temperature and pulse.

In sheep especially, the disease often causes fatal epidemics. In the mouse, *Sarcocystis muris* is a deadly parasite. Theobald Smith showed that gray and white mice may become infected with *Sar. muris* by eating infected mouse flesh containing motile sporozoites.

[1] This account is taken largely from Minchin's article on the Sporozoa in Ray Lankester, "A Treatise on Zoölogy," Part I, Second Fascicle. London, 1903.

Laveran and Mesnil claim to have extracted a toxin (*Sarcocystin*) by means of glycerin or salt solution, which they have found extremely toxic for experimental animals. (0.0001 gm. kills 1 kgm. of rabbit.) The dried and powdered extracts are also virulent. These extracts will remain virulent for a long time in the ice-box, but will not withstand heating above 60° for any time.

Darling (1909) describes a case of human sarcosporidiosis occurring in Panama, from which he studied the organism and came to the conclusion that it was probably a different species from the one already described as occurring in man. He gives a good historical review. Later he decides that morphologically his human sarcosporidia are identical with *Sarcocystis muris*.

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CHAPTER XLV.

THE MALARIAL ORGANISMS. BABESIA.

Introduction.—The malarial organisms are a group of protozoan parasites found to be the cause of a definite group of specific infectious fevers in man, called by the somewhat misleading term malaria, a term which signifies "bad air."

They are classed as sporozoa, order hæmosporidia, and are considered by the majority of observers as forming one genus, plasmodium.

Hartmann thinks that this group should be placed in the new order, binucleata, which he has created under the flagellata. He considers that they have lost by their endo-globular parasitism most of the characteristics of this order, but that in a few stages, he points out, they still show the flagellar and binucleate phases, two of the most important characteristics of this order.

So far as is known, the only means by which the malarial organisms are transmitted to man is mosquitoes of the genus anopheles. A part of the life cycle of the organisms is carried on in the body of these mosquitoes. The parasites develop in man within the red blood corpuscles which they finally destroy, thus producing the anæmia and pigment granules peculiar to malarial fevers.

Historical Note.—The fevers caused by these organisms were recognized and studied as early as 400 B. C., but it was not until 1880 that the true nature of the dancing pigment which had been observed long before was determined. At that time Laveran announced that he had discovered a parasite in the blood which he claimed was the cause of the disease and he published a good description of several of the stages in the life of the organism. The public remained at first almost entirely unconvinced of the parasitic nature of these bodies. Many still believed that the bacillus described shortly before by Klebs was the cause of the fevers. Among others, Marchiafava and Celli in Italy believed that Laveran's organisms represented areas of degeneration within the red blood cells, though Laveran himself demonstrated the organisms to them. When they began, however, to study the fresh tissue themselves they changed their opinion and later they published a number of valuable contributions on this subject. They gave the organism described by them the inappropriate name, *Plasmodium malariæ*. Laveran's researches were later confirmed by many other observers, and, though not all of Koch's laws have been verified in this case, the fact that a protozoan, the plasmodium, causes malaria is accepted as proved.

In 1885 Golgi showed that quartan fever depends upon a specific form of the parasite, and that the malarial paroxysm always coincides with the sporulation or segmentation of a group of parasites. Thus, in a single infection with the quartan variety a paroxysm occurs every fourth day, with a triple infection on successive days, segmentation with its accompanying paroxysm occurs daily. Golgi and others soon showed that tertian fever and æstivo-autumnal fevers were each due to a distinct variety of the plasmodium. These varieties are at present regarded by some as distinct genera.

by others as species, belonging to a single genus. Councilman first called attention to the diagnostic value of the different forms which appear in the blood.

Though it had been thought for nearly 2000 years that malaria is transmitted by insects, the question was not definitely settled until Ross in 1896 clearly demonstrated that the hæmatozoa of birds were transmitted by a certain species of mosquito. These investigations of Ross were soon confirmed by Grassi, Bignami, and others. MacCallum's observations on the sexual forms of halteridium were a great advance, and Bignami, Grassi, and others soon proved that all varieties of malarial fevers are transmitted from man to man by mosquitoes of the genus *Anopheles*. Grassi worked out the complete life cycle of the pernicious type (æstivo-autumnal), while Schaudinn (1901) did the same for the tertian form.

Materials and Methods for Study.—If a case of malaria is at hand the organism may be examined alive under the microscope by allowing a cover-glass to drop gently upon a drop of fresh blood placed upon a clean glass slide. For finer differential points, however, smears should be made. The making of these smears is a simple matter. There are the cover-glass and the slide methods, both of which have their peculiar advantages. To make a cover-glass preparation, two square, very thin (hence flexible) cover-glasses are cleaned. Holding one with thumb and index fingers by opposite corners, the tip of a drop of blood obtained by needle puncture of finger or lobe of ear is made to touch the centre of the cover-glass, and the second clean cover-glass held similarly is allowed to fall upon the first one in such a manner that the corners do not coincide. The blood droplet spreads by capillarity into a thin film, which is a sign to pull the two covers apart in the plane in which they lie; good results depend upon cleanliness, rapidity, and success in sliding the two covers apart.

A simpler way is to polish two slides. The tip of the exuded blood drop is made to touch one slide near one end and the edge of the second slide, held at an acute angle to the first one, is made to bisect the drop, which will spread at the point of contact by capillarity across the slide. Upon pulling the second or spreading slide over the first slide, never changing the angle and applying gentle pressure, a thin layer of blood suitable for examination will be formed. A slide made in this manner should be dried immediately by agitation in the air. It may then be fixed and stained in various ways. The following staining methods may be recommended:

Jenner's Stain.—Clear pictures of parasites, which, however, show no chromatin; hence unsuitable for study of finer differential points.

Nocht-Romanowsky Method.—Very suitable, but requires accurate mixture of several fluids just before using, which afterward have to be thrown away.

Wright's Stain.—Practically identical with Goldhorn's one-solution stain (*vide infra*), but less rapid; powerful chromatin stain and general blood stain.

Polychrome Methylene Blue (Goldhorn).—To prepare the stain dissolve 1 gram lithium carbonate in 200 c.c. clean water and add 1 gram methylene blue. Shake and dissolve. Pour into porcelain dish over water-bath, stirring frequently until blue color changes to a rich purple. Run through cotton in funnel; make up to 200 c.c. To 100 c.c. add 5 per cent. acetic acid until a faint pink is just visible on litmus-paper above level of point discolored by the dye. Now add the remaining 100 c.c. of dye and allow to stand in open dish for forty-eight hours. Run once more through cotton into clean bottle.

It is not necessary to use distilled water, and satisfactory results are obtained with all the different forms of methylene blue tried. B-X Gruebler is preferable.

Fix the smear by immersion in commercial wood alcohol for fifteen to thirty seconds; wash well and stain for about ten to fifteen seconds in polychrome; wash and stain for from fifteen seconds to sixty in $\frac{1}{20}$ per cent

aqueous eosin. Wash again in water and dry in air without heat. Body of parasites blue; chromatin is red to purple.

Results may be varied by using polychrome or eosin for different lengths of time. Admirable preparations may be obtained, even when there is precipitation, by just rinsing the smear a little in 50 per cent. ethyl alcohol. This will remove any precipitation.

The simplest method of staining the parasite is probably the following, recommended by Goldhorn for the staining of mast-cells: Saturate wood alcohol with methylene blue. Pour on dry smear for five to ten seconds and wash in water. Parasite blue.

Goldhorn's One-solution Stain.—To Goldhorn's polychrome methylene-blue (*vide supra*) add weak, watery (½ to ½₀ per cent.) eosin until the filtrate is of a pale blue color; the exact amount of eosin will depend upon the degree of alkalinity of the polychrome and upon the amount of unaltered methylene blue in the polychrome.

The precipitate is washed with water and dried without heat and protected from dust. When absolutely dry it is dissolved in commercial wood alcohol, making a 1 to 2 per cent. solution.

The smear is dried without heat and held for a second or two in the dye. It is then dipped slowly into a vessel with clean water, *film side down*; it should not be plunged into the water. The staining depends upon the interaction of the water with the film of dye adhering to the blood. Hold preparation in the water for a few seconds, then move it about for a moment, and rinse in clear water; clean lower side of the slide, as precipitation will have taken place here; hence, do not introduce into water with film side up. Dipping the preparation for a moment into 50 per cent. ethyl alcohol removes smudges and precipitate.

Giemsa's Method (see p. 624) gives excellent results.

Ross' Method of Examining a Large Quantity of Malarial Blood in One Film.—A large drop of blood (about 20 c.mm.) is placed on a glass slide and is slightly spread over an area which can be covered by an ordinary cover-glass. This is allowed to dry in the air or it is warmed over a flame without heating it more than enough to fix the hæmoglobin. The dry film is then covered with an aqueous solution of eosin (10 per cent.) and allowed to remain about fifteen minutes. This is then gently washed off and a weak alkaline methylene-blue solution is run over the film and left for a few seconds, when the preparation is again gently washed. After drying it is ready for examination.

The Parasite.—Three distinct species of malarial organisms in man have been described: *Plasmodium vivax* (causing tertian fever), *Plasmodium malariæ* (causing quartan fever), and *Plasmodium falciparum* (also known as *Laverania malariæ* and causing æstivo-autumnal fever). The last species has been divided by certain authors into two varieties, a quotidian and a tertian. On the opposite page is a table of the chief differences between these forms.

Each of these species undergoes the two phases of development already alluded to, one within the red blood cells of human beings (the asexual phase); the other within the digestive tract of the mosquito (the sexual phase). The form changes which the parasite undergoes throughout the whole cycle in both hosts are shown on Plate III, for the tertian parasite, which may be considered a type of all. Briefly, they may be described as follows.

The Asexual Cycle (Schizogony) Occurring in the Blood of Man.—The young form is often difficult to find in fresh blood. A pale area

TABLE SHOWING CHIEF DIFFERENCES BETWEEN THE SPECIES OF MALARIAL ORGANISMS (GENUS PLASMODIUM) FOUND IN MAN.

Name of organism.	Size of parasite up to segmentation, (Schizont).	Motion of young schizont in corpuscle.	Time of appearance of melanin granules and their arrangement.	Shape of segmenting parasite, number of segments, and site of segmentation.	Asexual cycle complete in	Sexual forms.	Incubation period.	Effect on human host tissues.	Remarks.
P. vivax (parasite of tertian fever).	1μ to slightly larger than normal red blood cell (may occasionally be almost twice size).	Markedly active.	6 hrs. First scattered then gathered in centre. Finely granular. Actively dancing.	Irregular mulberry. 12–24 (average 16). Peripheral circulation.	48 hours.	Gametocytes spherical. No crescents. Male $\frac{3}{4}$ size of female, which is 1$\frac{1}{2}$ times size of red blood cell.	About 14 days.	Pale, granular, slightly enlarged red blood cells. Finely granular pigment formed from metamorphosed hæmoglobin.	Double infection may cause a paroxysm every day, thus giving clinically a quotidian type of fever.
P. malariæ (parasite of quartan fever).	1μ to little less than size of red blood cell.	Not very active.	Within a few hours. Collected in zone on periphery. Coarsely granular. Slight dancing	Regular daisy shape. 6 to 14 (average 8). Peripheral circulation.	72 hours.	Gametocytes spherical. Fewer than in vivax. About size of red blood cell. No crescents.	About 3 weeks.	Red blood cells may be slightly shrunken.	Double infection may cause a paroxysm every day, thus giving clinically a quotidian type of fever.
P. Falciparum (parasite of æstivo-autumnal (pernicious) fever 1 quotidian type.	Smaller than others, from very small to $\frac{1}{2}$ diameter corpuscle.	Active, but slightly less so than tertian type.	Within 24 hrs. Small amount, 2 to 3 coarse granules usually central. Non-motile.	More or less symmetrical daisy. 6 to 8, very small. Chiefly in bone marrow and viscera.	24 hours.	Gametocytes crescentic, short and plump. $\frac{1}{4}$ size of red cell.	About 10 days.	Red blood cells greenish, shriveled (crenated) and darkened.	
2. Tertian type.	Larger than quotidian, about $\frac{1}{2}$ diameter of red blood cell.	Active, but less so than quotidian type.	Within 24 hrs. Small amount, but more than in quotidian type. Sluggish motion.	More or less symmetrical daisy. 10–24 (average 15). Chiefly in bone marrow and viscera.	48 hours.	Gametocytes crescentic, slender, with pointed ends.	About 12 days.	Same as quotidian form.	

is seen on an otherwise unaltered red corpuscle, situated usually eccentrically, about one-tenth the size of the red corpuscle or about one-fourth its diameter, when at rest presenting a rounded appearance, but usually actively amœboid, throwing out distinct pseudopodia, never remaining long in the same focal plane, frequently dipping, so to speak, into the substance of the corpuscle. It is often called the hyaline form because it is free from pigment, but it is not hyaline in the proper sense of the term. It is also called the ring form, because of its resemblance to a ring in stained preparations; but it is never a true ring. The ring appearance is produced by the formation of a large food vacuole. The young organism passes from the surface to the interior of the red corpuscles and grows there at the latter's expense,

The forms intermediate between this and the segmentation stage appear in the fresh blood simply as larger parasites, which are readily found on account of the reddish-brown pigment granules that they contain. These granules begin to appear several[1] hours after the organism has infected the red blood cell. At this time the organism is usually actively amœboid and the granules have a lively dancing motion, due to protoplasmic currents in the parasite. The infected corpuscle is swollen and paler, in forms other than tertian the infected red blood cells are smaller than normal.

When the parasite has approached nearly to its full growth, it occupies the greater portion of the corpuscle, which is now more difficult to make out. The pigment is still more evident, so that this form is therefore most readily found. At this stage amœboid movements are not so active. When full growth is reached, segmentation occurs. The forms up to the period of segmentation are called schizonts.

The morphologic changes which have been going on in the parasite preparatory to segmentation are best studied in properly stained smear preparations. In the living organism, they become presently sufficiently distinct to be followed; the pigment gathers more or less centrally into a compact mass, and a peripheral notching indicates that the parasite is preparing to divide into a number of segments called merozoites; the number of these segments varies in the different species. (See table.) Suddenly the segments separate as small spheroidal bodies, the young parasites. A corpuscular remnant and the pigment float away and are ultimately ingested by phagocytic cells. The young parasites attach themselves to red corpuscles as before and the human cycle is repeated (see Plate V for unstained organisms).

In a suitably stained preparation (any of the modifications of Romanowsky's stain, p. 597) the young parasite (see Plates VI and VII for the different species) appears to be a disk consisting of a central pale, unstained area, known as the achromatic zone, and of a basic (blue) periphery, the body, including a metachromatically stained, rounded, compact (red) chromatin mass, the nucleus, which tends to give the parasite the form of a signet ring.

[1] See table for number of hours in each species.

Later stages up to a certain number of hours show simply changes in size and outline of the body. The nucleus then divides by simple mitosis. Later it breaks up by amitotic division into an increasing number of small masses. By the time the chromatin division is completed the chromatin masses will have assumed a rounded form, and will be seen to exhibit ultimately the same strong affinity for certain dyes which is seen in the compact chromatin body of the young ring-like form. At this stage the heretofore scattered pigment appears in one clump. Good technique will always show a corpuscular remnant even at this time. The achromatic zone mentioned will be seen to develop with the chromatin, and when the next step, namely, the division of the body of the parasite, is seen to be completed, there will be as many achromatic bodies as there are chromatin bodies, each division having an equal share of the basic mother-body, each representing the young parasite (merozoite).

A certain number of the full-grown parasites do not segment and these are the forms which commence the life cycle in the mosquito. These forms grow to produce the sexual forms, the macrogametocyte, or female organism, and the microgametocyte, or male organism. When mature these forms are generally larger than the mature schizont of the same species, the female organism being usually larger than the male and containing more food granules and a smaller nucleus. In the æstivo-autumnal forms they are crescentic in shape, while in the other species they are spherical. In the circulating blood of human beings they show no further changes except to become freed from the corpuscle; but when the blood containing them is withdrawn and exposed for a short time to the air, an interesting series of changes in the microgametocyte is observed. The crescentic bodies are transformed into spherical bodies; the pigment of the microgametocytes becomes actively motile, due to internal agitation of the chromatin fibrils, which presently emerge as flagella-like appendages. Their movements are very rapid, causing corpuscles to be knocked about, and finally they become detached as the microgametes, or male elements, and go in search of the female element. In birds, one may actually observe the process of conjugation in slide preparations even without the aid of a moist chamber and heat. This transformation of male bodies never occurs in the human blood. It will be seen that it belongs to the sexual cycle which occurs in the stomach of the mosquito.

The Sexual Cycle (Sporogony) Developing in the Mosquito.—The common mosquito, often day-flying, belongs to the genus *Culex;* it cannot carry human malaria. It is easily distinguished from its night-flying or dusk-flying relatives, *Anopheles* (the malarial carrying mosquitoes comprise about eight genera of the sub-family anophelinæ), by its assuming a different posture on the perpendicular wall. While the *Culex* holds the body more or less parallel with the surface, the body of the *Anopheles* stands off at a marked angle. Other differential points are the following (see Fig. 190):

Wings of *Culex* are unspotted; those of *Anopheles* are spotted (except in one rare species).

The proboscis of *Anopheles* points toward the resting surface, while that of *Culex* does not do so.

Anopheles species bite usually in the early evening, while those of *Culex* bite almost at any hour of the day.

The male mosquito is readily told from the female by its plumed antennæ, those of the female being inconspicuous.

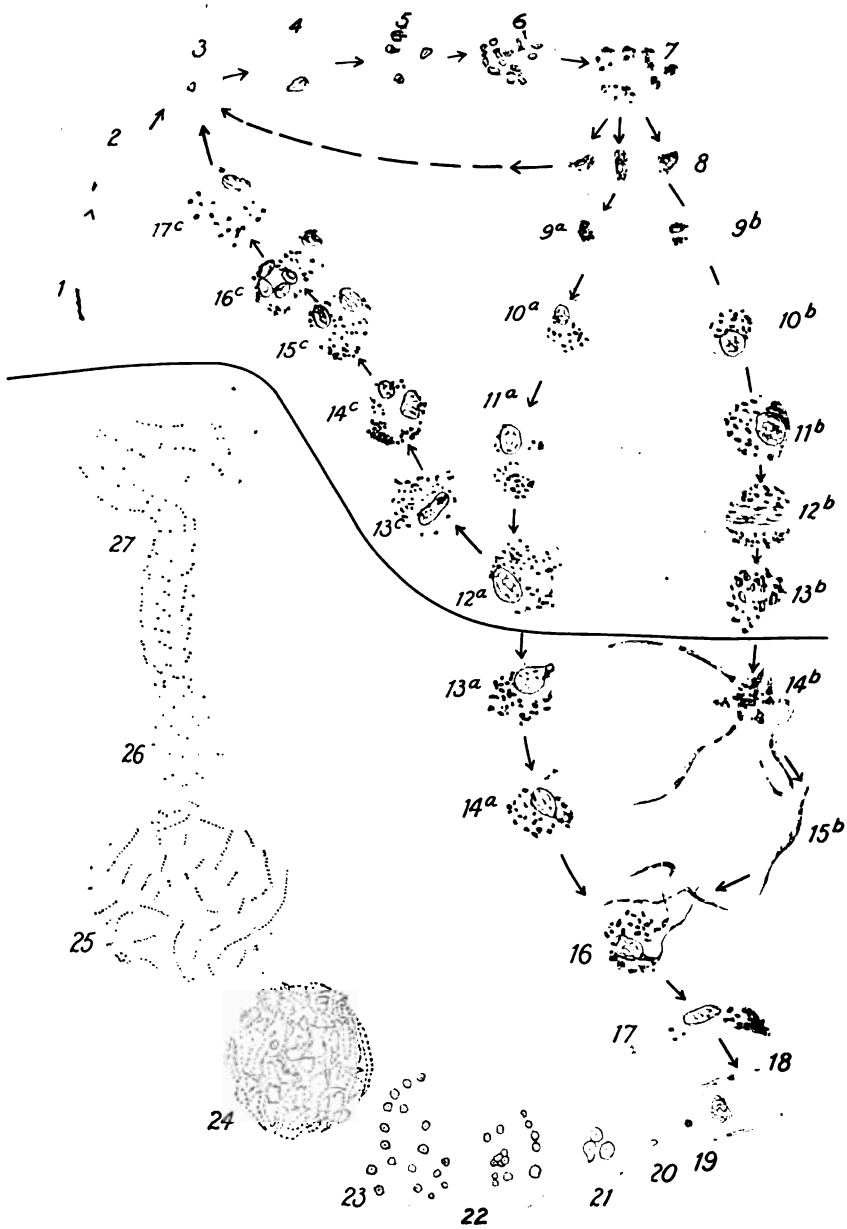
The eggs and the larvæ of the two genera are quite distinct as may be readily seen by glancing at Fig. 191. The anopheles mosquitoes breed in practically any kind of a collection of water, though some species prefer slow running water to quiet pools. The best known domestic carriers are usually found in barrels and cisterns.

If an ordinary mosquito (*Culex*) is allowed to imbibe the blood of a malarial patient whose blood shows gametocytes there will be simply a digestion of such blood in the mosquito, and no development of the malarial organisms results. If, however, certain species of *Anopheles* ingest such blood, immediate changes follow. It should be remembered that only female mosquitoes are blood sucking; hence, they alone can be responsible for the spreading of the disease. It should also be remembered that if the blood imbibed by the anopheles does not contain gametocytes, though it may contain earlier stages of the malarial organisms, no amount of such blood can cause general infection of the mosquito. The sexual cycle is similar in all species of the parasite.

The flagellation of the male parasite described above will promptly take place in the stomach of the anopheles, 4 to 8 microgametes being formed; these conjugate with the female element (Plate III) in a manner comparable to the impregnation of the ovum of higher animals by spermatozoids. The macrogametocyte becomes a macrogamete by the formation of a reduction nucleus which is thrown out of the organism (Plate III, Figs. 13*a* and 14*a*).

The product of conjugation, the oökinet (zygote), remains for a number of hours in the juices of the chyme stomach, changing gradually from a spherical, immobile body into an elongated wormlet endowed with motility (Plate III, Fig. 17). This penetrates the epithelial lining of the stomach and rests in the tunica elastico-muscularis (Plate III, Figs. 8–20); here it changes into an oval, then into a round body, which grows in the course of the next few days enormously, forming a cyst which projects into the body cavity. Meanwhile the chromatin will have become very active. It will have divided into numerous nuclei, which become arranged around inactive portions, and filamentous sporozoites develop from this chromatin and surrounding protoplasm (Plate III, Figs. 21–24). These sporozoites ultimately fill the cysts, which rupture, setting them free into the cavity of the mosquito's body (Plate III, Fig. 25); they then are carried by the lymph to all parts of the body of the mosquito and thus reach a glandular structure in the thoracic cavity of the insect, the so-called salivary

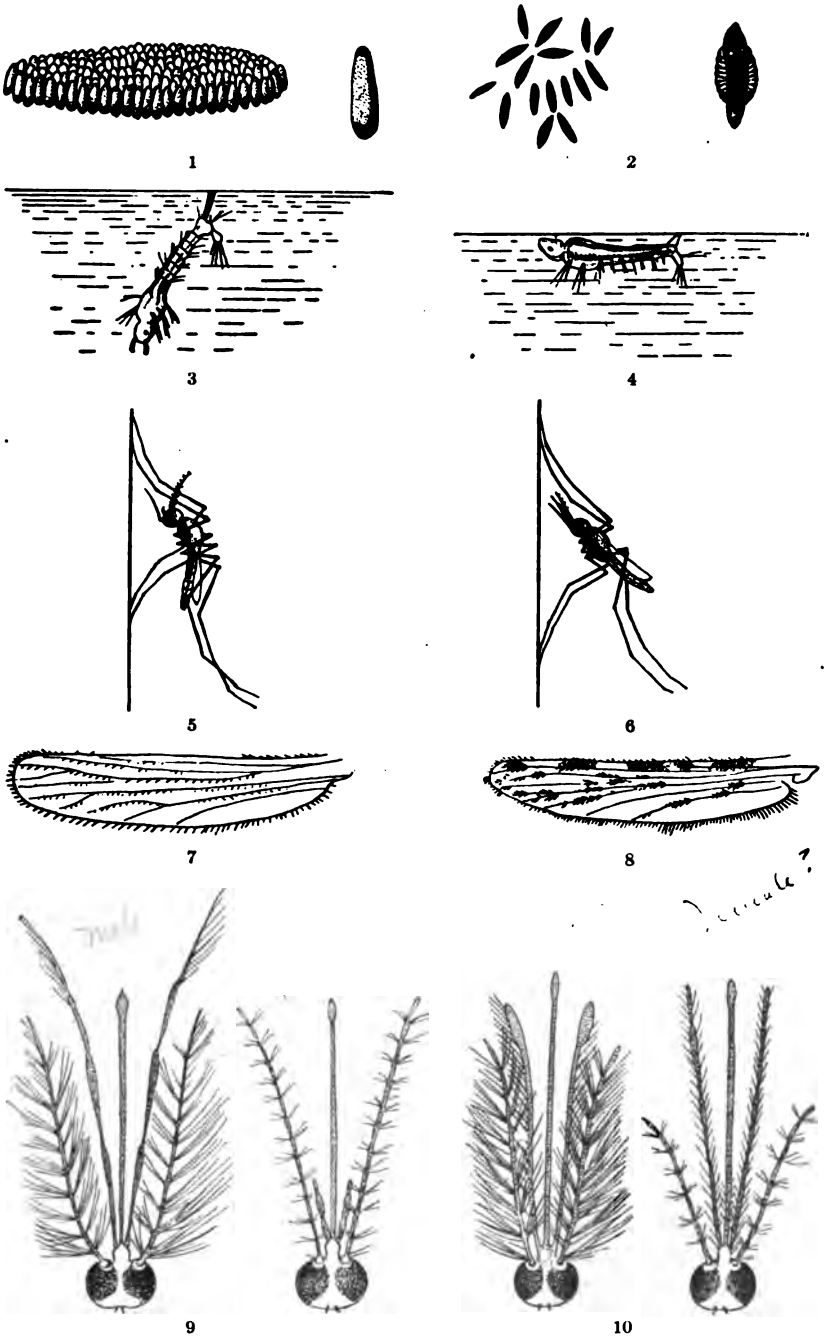
PLATE III



Life-cycle of Plasmodium Vivax. (After Grassi and Schaudinn.)

The human cycle is above the transverse line, somewhat rearranged by Kisskalt and Hartmann. The cycle in the mosquito is beneath. 1 to 7, schizogony; 1, sporozoite; 2, entrance of the sporozoite; 3 and 4, growth of the schizont; 5 and 6, nuclear division of the schizont; 7, formation of the merozoites; 8, merozoites; 9*a* to 12*a*, growth of the macrogametocyte; 9*b* to 12*b*, growth of the microgametocyte; 13*c* to 17*c*, parthenogenesis of the macrogametocyte; 13*a* and 14*a*, maturation of the macrogamete; 13*b* and 14*b*, growth of the microgamete; 15*b*, microgamete; 16, fructification; 17 oökinet; 18 to 20, entrance of the oökinet into the stomach wall of the mosquito; 20 to 25, sporogony; 22 and 23, nuclear multiplication in the sporont; 24 and 25, formation of the sporozoites; 26, passage of the sporozoites to the salivary gland; 27, salivary gland of the mosquito with sporozoites. (Magnification 1 to 17*c*., 1200 to 1; 18 to 27*c*., 600 to 1.)

FIG. 191



Chief comparative characteristics of *Culex* and *Anopheles*. (From Kolle and Hetsch.) Egg of *Culex* (1) laid together in "small boat," those of *Anopheles* (2) separate and rounded. Larva of *C.* (3) hangs nearly at right angles to water surface, those of *A.* (4) are parallel to surface. Body of *C.* (5) when resting is held parallel to wall in a curved position, that of *A.* (6) stands at an angle of about 45° and is straight; wings of *C.* (7) are generally not spotted, those of *A.* (8) are spotted; in *C.* the palpæ (9) of the female are very short, of the male are longer than the proboscis; in *A.* the palpæ (10) of both sexes are about equal in length with the proboscis.

gland (poison gland), in which they accumulate in large numbers (Plate III, Figs. 26–27). This gland is in immediate connection with the biting and sucking apparatus. If, now, such an infected mosquito "bites" a human being, the lubricating fluid of the puncturing apparatus will carry sporozoites into the latter's blood and the human cycle begins. The stages of development in the mosquito require from seven to ten days, but only when the temperature is favorable.

Effect on Man (Pathogenesis).—As the organism grows at the expense of the red blood cells the principal change is in the blood. Melanæmia, or the formation of pigment granules from the destroyed red blood cells, is one of the most characteristic features of malaria. As the disease progresses the red corpuscles show varying changes in form and hæmoglobin content, not only the infected corpuscles, but others as well, thus showing that the organism produces either primarily or secondarily some toxic substances. The pigment occurs in two forms, melanin and hæmosiderin. The second only gives the reaction for iron and is found in the internal organs, while the first is found everywhere in the circulating blood. The pigment is taken up by the leukocytes. There is usually a definite reduction of both red and white blood corpuscles, which is more marked in tertian and quartan malaria than in æstivo-autumnal. There is a relative increase in the number of mononuclear leukocytes. The spleen shows marked hyperplastic inflammation and pigmentation.

After death, which sometimes takes place in cases of pernicious æstivo-autumnal fever, there are scattered areas of intense congestion and of parenchymatous inflammation in the various internal organs, together with the presence of large numbers of the parasite.

Toxin Production.—The relationship between segmentation and paroxysm is always noted in tertian cases, and it is reasonable to suppose that the occurrence of the paroxysm is referable entirely to the liberation of toxic substances resulting from metabolic activity of the parasite within the corpuscle. That there should be a toxic product seems highly probable, and its amount must be considered in heavy infections. Cases showing an infection of 1 to 5 per cent. of all corpuscles are not infrequent; the destruction of from 50,000 to 200,000 or more corpuscles per cubic millimetre of blood leads to the rapid deglobularization of the blood; hence the deficiency in numbers; add to this the effects of the metabolic products, and little is left to the imagination to explain the pronounced anæmia.

Immunity from malaria appears to exist as natural and acquired immunity.

Prophylaxis.—The fact that, with the extermination of the malarial carrying mosquitoes, malarial fevers in man would be made impossible, remains established; the parasite must have its chance of rejuvenescence in the mosquito's stomach.

The various methods of extermination are fully described in books which go minutely into the subject. The method of giving small doses of quinine to human beings exposed to *Anopheles*, and of thus getting rid of the organism itself within man, should be considered. In hot climates especially, where it is practically impossible totally to

Description of Plate IV.

1. Typical young tertian form; the corpuscle shows incipient degeneration; corpuscle to left above shows a blood platelet.

2. Abnormal young form, showing small accessory chromatin body.

3. Two parasites; one a normal young form; the second a large form in crenated corpuscle is an unusual abnormal form with very large achromatic area.

4, 5, 6. Estivo-autumnal parasites; single, double, and triple infection; central elongated chromatin bodies. These forms are about the largest usually seen in the peripheral blood; no degeneration of corpuscle.

7. Tertian parasite, about ten hours old; marked degeneration of corpuscle.

8. Double infection of a corpuscle in tertian fever; marked degeneration of corpuscle.

9, 10, 11. Large tertian parasites showing division of chromatin previous to segmentation.

12 and 14. Complete segmentation of tertian parasite.

13. Double infection of corpuscle, one parasite reaching maturity, but showing unusually small segments; the second one atrophied.

15. Tertian parasite, old case; while the parasite is only half-grown, the chromatin has split into several compact masses. Degeneration of infected corpuscle.

16. Dwarfed tertian parasite, smaller than a red corpuscle, but showing five compact chromatin bodies; resemblance to quartan rosette.

17. Microgametocyte of tertian malaria; prominence of blackish pigment surrounding a large achromatic zone in which the microgametes lie coiled up.

18. Tertian macrogametocyte.

19 to 23. Crescentic bodies of estivo-autumnal malaria.

19. Typical gametocyte; pigment surrounding achromatic area; no chromatin shown: the "bib" is present. (Male?)

20. Semiovoid gametocyte. (Female?)

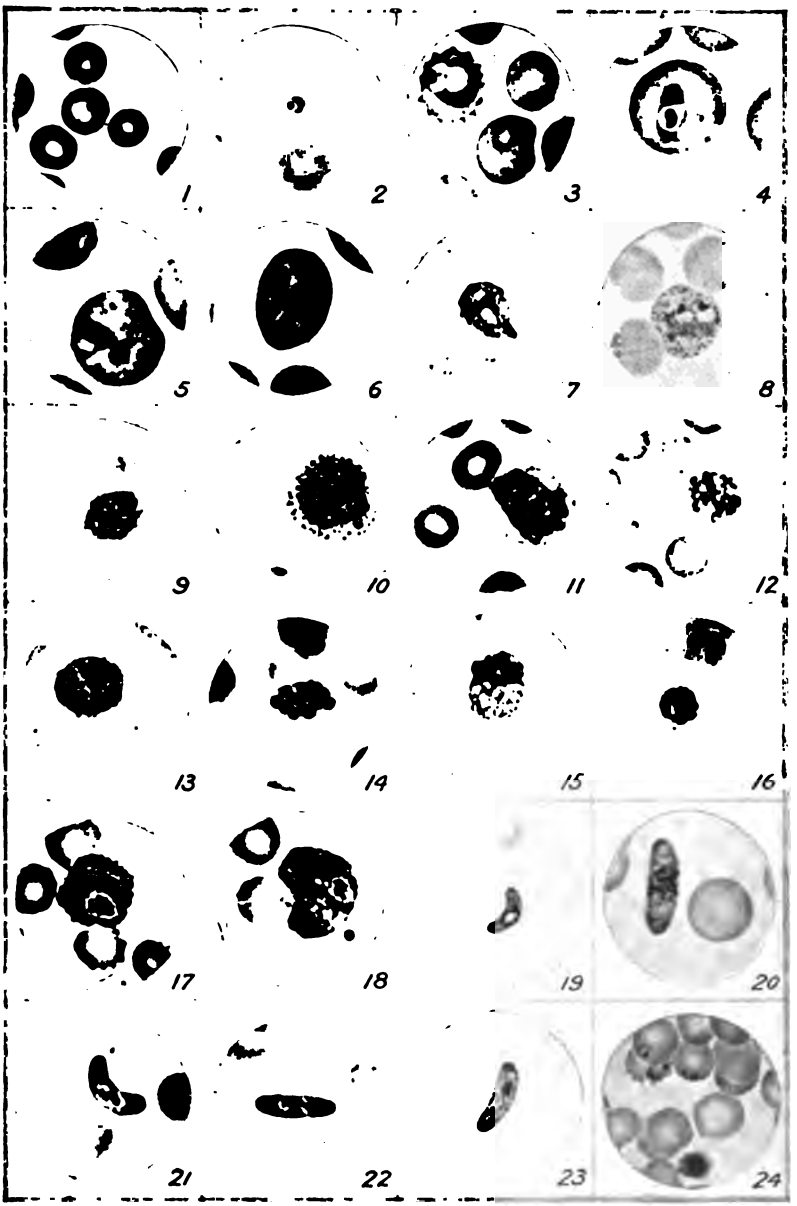
21. Pigment removed. Elliptical achromatic area in which the microgametes are seen.

22 and 23. Pigment removed; chromatin more compact; possibly female elements.

24. From a case of pernicious malaria with rich infection; only hyaline forms in peripheral blood. Below, a large blood-platelet.

Note.—As the amplification is not uniform, a comparison of the parasites with the blood corpuscles shown should be made in order to have a correct conception of their size.

PLATE IV



Photographs of Tertian and Estivo-autumnal Malarial Parasites in Different Stages of Development. (Goldhorn.)

destroy the breeding places of the mosquitoes, this method is especially serviceable.

Points in Diagnosis.—By a study of the circulating parasite the examiner should be able to tell not only the species present, but also the progress the disease is making. Malarial parasites can always readily be found in recent primary infections, and it is usually only in old cases that the search becomes difficult; one is, however, generally rewarded by finding them if one looks long enough for them.

A helpful sign is the finding of pigment in mononuclear leukocytes, which are seen about the time of a chill or of the period symptomatically corresponding to it. Free pigment cannot be used as a means of diagnosis, as it may be impossible to tell it from dirt or dust. In a primary infection of *long standing*, the gametocytes may be found, and in relapses and in those cases treated by quinine, many atypical forms appear. A small dose of quinine may drive all parasites except the sexual forms out of the peripheral circulation; at all events, the finding of them becomes, in the absence of gametocytes, a matter of time and experience, especially also as they may be much altered in appearance. The part most and first affected is the blue staining body; later follow eccentricities of the chromatin, such as multiple bodies, and dwarfing, just such changes as might have occurred in time, if the body had been allowed to combat the parasite without the aid of drugs. In both cases the fever curve becomes atypical. It should be remembered that there is no quotidian form originating in this country. Quotidian paroxysms occurring here are either a double tertian or a triple quartan infection. The notion that the parasites can be found only at the time of the paroxysm is still in the minds of many; it is erroneous. The gametocytes are quite resistant to quinine and other drugs, and it appears as if cases in which these forms are seen are much more prone to relapse than promptly treated recent primary infections. The macrogametocytes may remain quiescent for years in the blood, and then under certain conditions, probably through parthenogenesis, may again begin to develop and multiply, thus bringing about relapses.

In the æstivo-autumnal forms the crescentic gametocytes are generally few, but at times large numbers of them develop. Of course, they are absolutely characteristic. The young parasites are more or less characteristic in stained preparations (Plate VII). There may be as many as seven parasites in one corpuscle. Later the few heavy pigment granules are characteristic.

In fatal cases the formation of crescents may not take place; the blood infection with young parasites is then enormous, every field of the microscope showing numbers of them.

In the study of æstivo-autumnal fever as well as in that of the other forms, it is to be remembered that crescents when found indicate that the disease is of some standing, for such sexual forms are not formed until the asexual propagation is waning. The recognition of these ovoidal and crescentic bodies is easy. But as there are no readily

discoverable pigmented forms in the peripheral blood in the early stages, it is necessary to be thoroughly familiar with the young æstivo-autumnal forms. Polychrome staining for them cannot be too much recommended, as there is little that is characteristic about them when they have been stained with methylene blue alone. Many a serious error has been made by adhering to the antiquated idea that parasites should be looked for in the fresh blood, as these young, non-pigmented, so-called hyaline forms cannot be readily recognized by the inexperienced, while it is an easy matter to know and classify them when properly stained.

The recognition of the quartan parasite in its early stages in the fresh blood is not as difficult as that of the tertian form, because the outline is more distinct; but in stained preparations it is often indistinguishable from the latter. The living amœboid young form or schizont is more refractive than the young living tertian schizont, more like the æstivo-autumnal form, and it is just as sluggish in its movements. Here, too, the corpuscle is often shrunken and looks as if it contained more hæmoglobin than in the case of infection with the tertian parasite.

The growing parasite rapidly becomes pigmented, but it shows fewer, larger, less motile pigment granules than the corresponding tertian one; moreover, the pigment is arranged around the periphery of the organism, while in the tertian form it is distributed throughout the protoplasm. (Plate II, Fig. 13 *a* and *b*.) The quartan parasite is apt to form a band across the infected corpuscle. Segments are few in number, as a rule, and the parasite remains dwarfed, while the infected red blood cells are normal in size. The segments are generally arranged symmetrically around the central pigment, giving the so-called daisy or marguerite appearance to the parasite at this stage.

In tertian fever, the granular degeneration which the infected corpuscles early undergo is diagnostic. In the first few hours it resembles the ordinary granular stroma degeneration with basic affinity, while it is later seen that the affinity of the then more numerous granules is more acid, or at least the staining is no longer orthochromatic, the blue being superimposed by a red; in other words, these granules stain later metachromatically. The greater the loss or transformation of the hæmoglobin, the greater the number of granules. This holds good only for tertian parasites, the æstivo-autumnal variety causing practically no appreciable change though the same technique be used.

Malarial-like Parasites in Other Animals.—Two genera of protozoa closely related to the malarial organisms have been found in birds: (1) the proteosoma or hæmoproteus; (2) the halteridium; both found in owls (*Hæmoproteus noctuæ* Celli and Sanfelice). Points in their life history have been brought out by various observers, especially by Ross and by MacCallum. The complete life cycle of both forms, as worked out by Schaudinn, is considered by him and his followers to be of fundamental importance to the understanding of the re-

lationship of blood parasites. Schaudinn states that these organisms pass through a flagellate stage in the intestinal tract of the common mosquito (*Culex pipiens*) which had previously fed on owls infected with the intracellular organisms (halteridium and hæmoproteus). Novy considers that this mosquito flagellate stage of Schaudinn is simply a growth of trypanosomes in the mosquito's intestinal tract which are normally found there, and that Schaudinn did not sufficiently control his work to warrant his conclusions.

Malarial-like organisms have been found also in monkeys, cattle, dogs, and frogs, but they have been little studied.

An interesting article by Bernberg-Gossler on the malarial organisms in monkeys has just appeared. In it the author describes a binucleate phase of these plasmodia and agrees with Hartmann in his recent classification of these organisms (see p. 596).

GENUS BABESIA (PIROPLASMA).

It was not until 1888 that there was a hint as to the real nature of the actual cause of "Texas fever" (bovine malaria, tick fever, hæmoglobinuria) and allied diseases which attack field cattle in many parts of the world. Then Babes described inclusions in red blood cells in Roumanian cattle sick with the disease, though he did not decide upon the nature of the organism. No new studies were reported until 1893, when Theobald Smith and Kilborne gave such a complete description of this disease and its cause as occurring in Texas cattle that little that is new has since been discovered.

These authors describe as the cause of Texas fever, pigment-free ameboid parasites appearing in various forms within the red blood cells of infected animals. The organisms may be irregularly round and lie singly or they may be in pear-shaped twos, united by a fine line of protoplasm.

Because of these double pear-shaped forms Smith and Kilborne named the organism *Pyrosoma bigeminum*[1] and they placed it provisionally among the hæmosporidia. These authors also showed that the contagion was carried by a tick (see below). Their work has been corroborated by many investigators in different parts of the world. Hartmann places this genus in his new order Binucleata, and he considers it an important form for showing the relationship of the endocellular blood parasites to the flagellates. Schaudinn, in 1904, was the first to call attention to the occurrence of nuclear dimorphism in *B. canis* and *bovis*, and Luhe, Nuttall and Graham-Smith, Breinl and Hindle and others have confirmed this observation. The second

[1] The generic name *Pyrosoma*, already in use for a well-known Ascidian genus, was altered to *Piroplasma* by Patton in 1895. In the meantime Starcovici (1893) had given the name *Babesia bovis* to the form described by Babes; and as this form seems to be identical with that described by Smith and Kilborne the correct name of the genus should be *Babesia*, while the species parasitic in cattle should be called *Babesia bigemina*.

nuclear mass is generally in the form of a small granule similar to the blepharoplast of undoubted flagellates.

Morphology of the Parasite (Plate II, Fig. 14).—In the examination under 1000 diameters of fresh blood of sick cattle, according to Smith and Kilborne, are seen, in the red blood cells, double pear-shaped forms and single rounded or more or less irregular forms. The size varies, though generally it is the same among the bodies in the same red blood cell. The average size is 2μ to 4μ long and $1\frac{1}{2}\mu$ to 2μ wide. The pointed ends of the double form are in apposition and generally touch, though in unstained specimens a connection between them cannot be seen. The axis forms either a straight line or an angle. The protoplasm has a pale, non-granular appearance, and is sharply separated from the protoplasm of the including red blood cell. The small forms are generally fully homogeneous, whereas the larger ones often contain in the rounded ends a large rounded body, 0.1μ to 0.2μ in size, which is very glistening and takes a darker stain. Within the largest forms in the centre of the thick end is a large round or oval body, 0.5μ to 1μ, which sometimes shows ameboid motions. Piana and Galli-Valerio (1895 and 1896) and other observers have since described definite ameboid motion of the whole parasite. The motion of the whole parasite on the warm stage is not produced by the formation of distinct pseudopodia, but by a constant change of the boundary. The changes can succeed each other so quickly that it is scarcely possible to follow them with the eye. The motion may persist for hours. The single ones show motion, while the double ones remain unchanged. The parasites take most basic aniline stains well. The Romanowsky method or its modifications gives the best results.

Stained by this method, the smallest forms appear as tiny rings, about one-sixth the diameter of the red blood cell. A part of the rim takes the red nuclear stain, the rest is blue. In the large mature pear-shaped organisms a loose mass of chromatin is at the rounded end and a dense, compact mass is situated nearer the pointed end. These mature, pear-shaped forms, Nuttall states, are the mark of distinction between *Piroplasma* (Babesia) and other intracorpuscular blood parasites. These pyriform bodies are generally present in pairs, and occasionally, in the acute form of the disease, sixteen pairs may be seen in a single blood cell.

The number of red cells infected is about 1 per cent. of the whole. If the number increases to 5 per cent. or 10 per cent. it generally means the death of the animal. The parasites quickly disappear from the blood after the disappearance of the fever. In fatal cases many parasites are found in the red blood cells of the internal organs. They vary in number according to the stage at which death occurs, are most abundant in the kidneys (50 to 80 per cent. of all red corpuscles infected), and are found in fewer numbers in the liver, spleen, and other internal organs.

R. Koch has described a bacillar form which he found in large

PLATE V

Fig. 1.—Tertian Malarial Plasmodium.

1. Hyaline form.
2. Pigmented ring form.
3 to 6. Pigmented forms.
7. Segmenting forms.
8. Flagellate form. (Microgametocyte.)
9. Non-flagellate form. (Macrogamete.)
10. Segmenting form after destruction of red corpuscle.

Fig. 2.—Quartan Malarial Plasmodium.

1. Hyaline forms.
2 to 5. Pigmented forms.
6 and 7. Segmenting forms.
8. Segmenting forms after the destruction of red corpuscle.
9. Flagellate form. (Microgametocyte.)
10. Non-flagellate form. (Macrogamete.)

Fig. 3.—Tertian Æstivo-autumnal Malarial Plasmodium.

1 and 4. Hyaline ring form.
2, 3 and 7. Pigmented ring form.
5 and 6. Pigmented forms.
8 Young intracorpuscular crescent.
9. Segmenting forms.
10. Flagellate form. (Microgametocyte.)
11 to 14. Crescentic forms.

Fig. 4.—Quotidian Æstivo-autumnal Malarial Plasmodium.

1 to 4. Hyaline ring forms. Some cells show infection with more than one organism.
5 to 7. Pigmented forms. In 6 one hyaline form.
8. Segmenting forms. Segmentation complete within infected red blood corpuscle.
9. Flagellate form. (Microgametocyte.)
10, 11, 13 and 15. Crescentic forms.
12. Ovoid form.
14. Non-flagellate forms. (Macrogamete.)

NOTE.—Mark the larger size and greater amount of pigment in the tertian æstivo-autumnal plasmodium.

PLATE VI

Fig. 1.—Tertian Malarial Plasmodium. Stained by Oliver's Modification of Wright's Stain.

1 to 4. Ring forms of tertian parasite.
5. Ring form. (Conjugation form of Ewing.)
6 to 10. Pigmented organisms.
11 to 14. Nearly full-grown forms, showing diffusion of the chromatin.
15 to 17. Segmenting forms within red corpuscle.
18. Segmenting forms after destruction of red corpuscle.
19. Flagellum. (Microgamete.)
20. Sporozoite.

Fig. 2.—Quartan Malarial Plasmodium. Stained by Oliver's Modification of Wright's Stain.

1 to 4. Ring forms of quartan parasite.
5, 6, 7, 8, 9. Pigmented parasites.
10 to 12. Segmenting forms of quartan parasite.
13. Segmenting stage after destruction of red corpuscle.

NOTE.—Chromatin of nucleus stained red; protoplasm stained blue; vesicular portion of nucleus unstained.

PLATE VII

Æstivo-autumnal Malarial Plasmodia. (Tertian.) Oliver's Modification of Wright's Stain.

1, 3, 4, 5, 6, 7, 8, 9, 10 and 15. Ring forms of tertian æstivo-autumnal plasmodium.
2. Intracellular form.
11, 13, 14, 16 and 17. Pigmented ring forms.
12. Red corpuscle showing infection with two "ring forms."
18 and 19. Pigmented forms, just prior to segmentation.
20, 21, 23 and 24. Round and ovoid forms developed from crescents.
22. Macrogamete.
25 to 36. Crescentic forms of æstivo-autumnal plasmodium (tertian).
29. Ovoid form.
37. Segmenting form.
38. Sporozoites.
a. Segmenting form of quotidian æstivo-autumnal plasmodium.

NOTE.—In this plate the tertian æstivo-autumnal plasmodium is shown. The staining reactions of the quotidian plasmodium are exactly similar.

PLATE V

FIG. 1

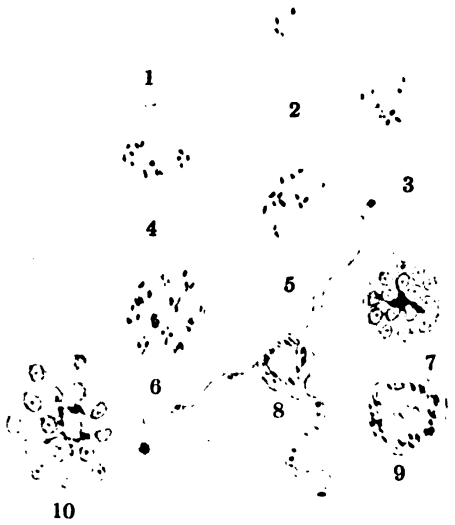


FIG. 2

FIG. 3

FIG. 4

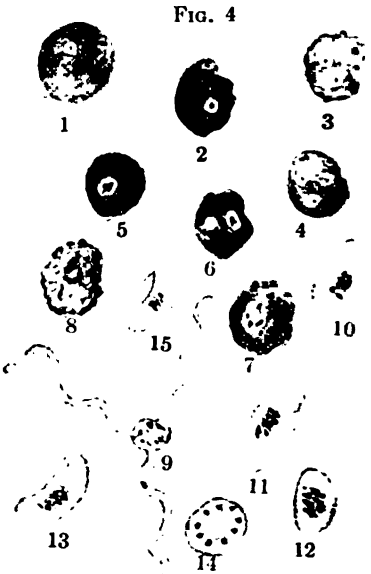


PLATE VI

FIG. 1

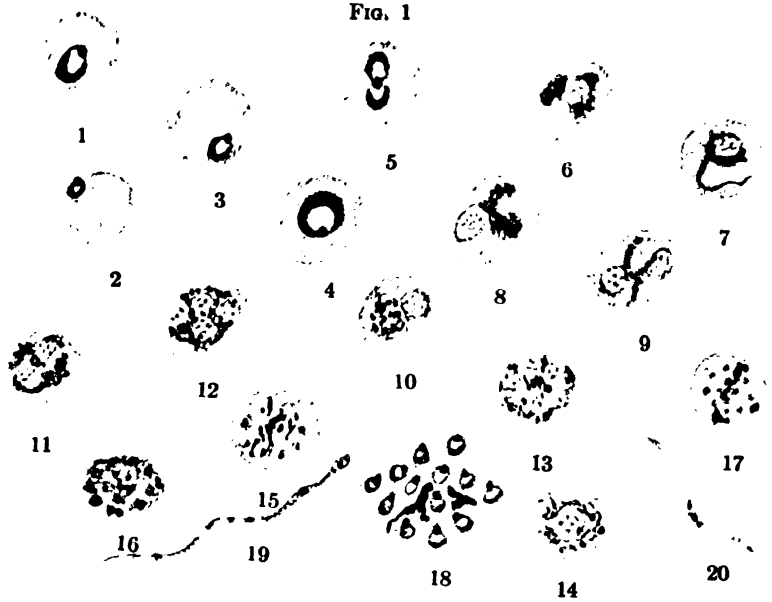


FIG. 2

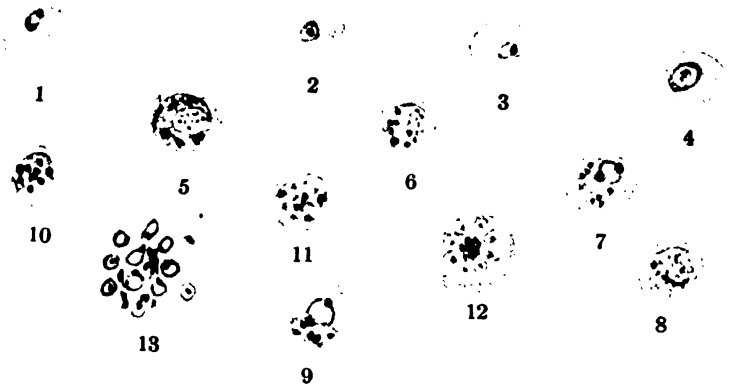
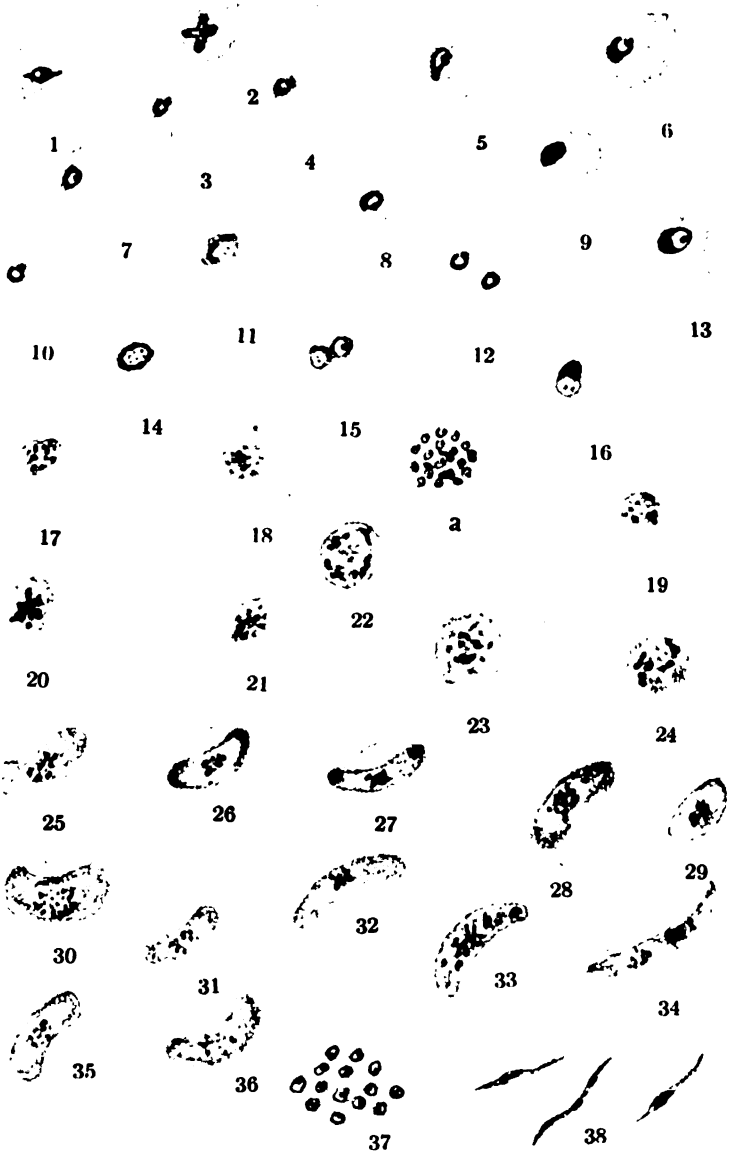


PLATE VII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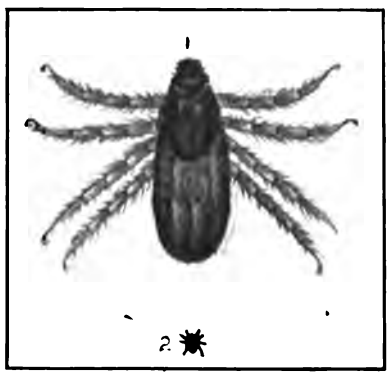
numbers in red blood cells of acute fatal cases in East Africa. Between these and the pear-shaped forms he found all grades. This variety is probably a distinct species.

Flagella-like appendages in *Babesia* have been discribed by several observers as occurring in the blood in mammals. More frequently they have been seen in the tick and in attempted cultures. Some of them have been interpreted as possible microgametes (Hartmann, Calkins), others as true flagella (Breinl and Hindle), still others as fine pseudopodia (most observers).

Smith and Kilborne showed that the infection is caused by a species of tick, *Margaropus annulatus*, Say (*Boöphilus bovis*) (Fig. 192), and

FIG. 192



No. 1. Texas fever tick, *Margaropus annulatus* (Boöphilus bovis), ×15.
No. 2. Natural size (Mohler).

Kossel gives *Ixodes redivius* as the tick causing transmission of the germ in the hæmoglobinuria of Finland cattle.

The ticks feeding upon the blood of cattle and other mammals become sexually mature at their last moult. They then pair, and the fertilized females, after gorging themselves with the blood of their host, drop to the ground. Each female then lays about 2000 eggs, and within the shell of each egg a large quantity of blood is deposited to serve as food for the developing embryo. The female then shrivels up, becoming a lifeless skin. The newly hatched larvæ containing in their abdomens some of the mother-blood, crawl about until they either die from starvation or have the opportunity of passing to the skin of a fresh host. If the mother-tick has drawn its supply of blood from cattle infected with piroplasma, her larvæ are born infected with the parasite and become the means of disseminating the disease further. This mode of dissemination explains the long incubation period of the disease (forty-five to sixty days—thirty days for the development of the larvæ and the remainder for the development of the parasite within the host). It is possible that the tick embryo acquires the infection secondarily from the

blood it absorbs in the egg, and that the parasites do not pass through the ovum itself as in *Nosema bombycis*. This species of tick *M. annulatus* has been found also on sheep and ponies.

So far, it has not been possible experimentally to inoculate animals other than cattle with these parasites. Calves withstand the infection better than older animals and a certain degree of immunity is reached in some of the older cattle in infected districts. The piroplasmata taken in by such animals may remain as harmless parasites for some time. If, however, such cattle are weakened from any cause, their resistance to the organism may be lowered and they may therefore pass through a more or less severe attack of the disease.

Symptoms of the Disease.—Fever (40° to 42° C.), anorexia, weakness, increased pulse and respiration, decreased secretion of milk, hæmoglobinuria at the height of the fever, causing the urine to appear dark red like port wine or darker. The urine may contain albumin even if the hæmoglobinuria is absent, but there are no red blood cells present, the color being due to the coloring matter of the blood only. There is icterus of the mucous membrane if much blood is destroyed.

The prognosis varies in different epidemics from 20 to 60 per cent. Death may occur in three to five days after first symptoms appear. Recovery is indicated by a gradual fall of the fever.

Treatment.—Quinine in large doses seems to have helped in some epidemics. Nuttall, Graham-Smith, and Hadwen have reported curative effects from trypanblau in both canine and bovine babesiosis (Piroplasmosis).

Prophylaxis.—Stalled cattle are not infected, but it is impracticable to keep large herds of cattle stalled. If the cattle are kept from infected fields for one or two years and other animals (horses and mules) are allowed to feed there the ticks may disappear. The burning of the field for one season may have a good effect. If animals cannot be taken from infected fields such fields should be enclosed.

Ticks on animals may be killed by allowing the cattle to pass through an oil bath (paraffin, cottonseed oil, etc.), whereupon the ticks die from suffocation. The bath should be repeated after a week in order to kill any larvæ which may have developed. All animals sent from infected regions should receive this treatment. Animals apparently healthy before the treatment, after the disturbing influence of the bath often develop the disease in an acute form and die.

Certain birds in Australia seem to feed on the ticks, therefore such birds might be propagated.

Various attempts have been made to give protection by the inoculation of fresh (not older than two or three days) blood from slightly infected animals. Some partial results have been reported, especially when the inoculations were made during the cold months. In Australia, the inoculation of defibrinated blood from animals which have just recovered from the infection, but whose blood still contains some parasites, has been tried. So far no absolute protection has been produced, neither does the parasite-free serum of animals which have entirely recovered from the disease seem to contain protective qualities.

Blood Organisms.—Blood organisms similar to those described in the hæmoglobinuria of cattle have been found in cases of red water fever of cattle in England. They also occur in monkeys, dogs, sheep, horses, and pigeons. Nocard and Motas, who have made an extensive study of these parasites in the malignant jaundice (hæmoglobinuria, malaria, or biliary fever) of dogs, state that though the parasites are morphologically similar to those infecting cattle, yet it is impossible to infect cattle or any other animal tried with them. They must therefore be considered a physiologic variety.

Nuttall and Grahâm-Smith have recently completed a series of articles in which they have reported a minute study of canine piroplasmosis, and have drawn a cycle showing the usual mode of multiplication in the circulating blood. They consider B. canis a species distinct from B. bovis and B. pitheci (found by Ross, in 1905, in blood of a species of cercopi-thecus) though no morphologic differences are given.

Christophers has described probable sexual stages of development in the tick *R. sanguineus*, so that he has drawn a complete life cycle of the organism. Various attempts at artificial cultivation have not met with much success.

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CHAPTER XLVI.

SMALLPOX AND ALLIED DISEASES. SCARLET FEVER. MEASLES.

SMALLPOX (VARIOLA) AND ALLIED DISEASES.

Introduction.—The diseases smallpox, cowpox, vaccinia, horsepox, sheeppox, if not identical, are closely allied. Indeed, the following facts seem to prove that at least cowpox and variola are very closely related, if not essentially the same disease: *First*, smallpox virus inoculated into calves produces, after passage through several animals, an affection exactly similar to cowpox. The successful inoculation of the first series of cattle from smallpox is a matter of great difficulty, but so many experimenters have asserted that this has been done that there seems to be no doubt as to its truth. In our laboratory not one of many attempts to accomplish it has been successful. *Second*, both when occurring in nature and when produced by experiment the lesions of the two diseases are similar. *Third*, monkeys have been successfully protected against either disease by previous inoculation of the other; also, observations go to show that human beings inoculated with cowpox vaccine are not susceptible to inoculation with smallpox virus, and that those who have within a varied time passed through an attack of smallpox cannot be inoculated successfully with cowpox vaccine. These facts seem positively to prove that the two diseases are produced by organisms originally identical, one being modified by its transmission through cattle, the other through human beings.

Variola is perhaps the most regularly characteristic of the diseases of man. It is highly infectious and is controlled only by vaccination. Notwithstanding the fact that we know definitely the exact site of the infective agent in this disease and that certain experimental animals are susceptible to inoculation of the material containing the infective agent, most investigators are still undecided in regard to the nature of the chief exciting factor. A few, however, claim that certain bodies found chiefly in the epithelial cells of the skin and mucous membranes in the specific lesions are protozoa causing the disease.

Definition.—Smallpox (Synonyms: Variola, la variola, Blattern, Pocken, Vajuola) is an acute infectious disease characterized by an epidermic eruption of vesicles and pustules which, upon healing, produce cicatrices of varying extent and depth.

Historical Note.—The first undoubted description of the disease was given by Rhazes in the tenth century, but it is evident that he did not consider it a new disease. To trace its original home seems to be impossible. It may have developed first in certain regions in Asia and Central Africa

where it is at present endemic and is said to be uncontrolled by vaccination. Many outbreaks of the disease in the United States can be traced directly to the importation of African negroes.

The disease, carried by the intercommunication, principally of war and commerce, was widespread when Edward Jenner showed conclusively in 1798 that vaccination with cowpox afforded protection. Now the few cases of variola that occur are seen in those who, through neglect or ignorance (sometimes willful), have not been vaccinated.

Etiology of Variola and Cowpox.—It has been repeatedly shown that no bacteria similar to any of the known forms have a causal relation to these diseases. In our own laboratory we are able, by the inoculating of rabbits' skins, to produce extremely active vaccine virus in large quantities, absolutely free from microörganisms which grow under the conditions of our present methods of bacterial cultivation. Such pure active vaccine, when emulsified in equal parts of glycerin and water and filtered through two or three thicknesses of the finest filter-paper, gives a slightly opalescent filtrate, which in the hanging drop under high magnification shows many very tiny granules with an occasional larger one, and in smears shows no formed elements giving characteristic stains. This filtrate, from which no growth can be obtained on artificial culture media, when rubbed over a freshly shaved rabbit's skin after the method of Calmette and Guérin, or when used to vaccinate human beings, gives an abundant typical reaction.

These facts show that some, at least, of the infective forms cannot as yet be made to grow outside of the body, that such forms are very tiny, and that they do not stain characteristically with our usual methods of staining. In a few experiments we were unable to filter the virus through a Berkefeld filter under forty pounds' pressure, but this may have been due to the fact that we did not dilute our virus sufficiently. Since then Bertarilli has reported moderately successful results from his filtration experiments.

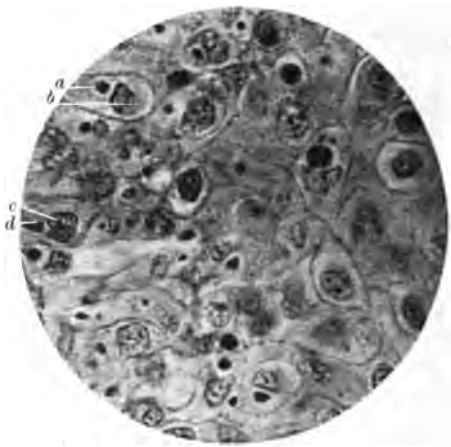
Since Guarnièri in 1892 claimed that certain inclusions present in the epithelial cells of the lesions of smallpox in a rabbits' cornea (Fig. 192) were parasites, much attention has been given to the study of these bodies, commonly known as "vaccine bodies," yet opinions still differ as to their nature. The most recent important studies of these bodies have been made, on the one hand, by Councilman and his associates, who believe them to be protozoa, and, on the other, by Ewing, who believes that all of the forms so far described are degeneration products, some specific, others not.

Councilman believes that there are two cycles of development of the "parasite," one intracellular and the other intranuclear, and that the intranuclear infection occurs only in smallpox. The intracellular cycle is simple, showing only "multiplicative reproduction," while the intranuclear cycle is more complicated, probably sexual in character. Calkins, working with Councilman, described a cycle of development in which we believe are included many forms due to degeneration of the

host cells alone. Calkins now thinks that his original tentative cycle was too elaborate. He still firmly believes that the bodies are protozoa, but that they belong among the rhizopoda and not among the microsporidia where he first placed them.

Prowazek and others believe that the organisms of this group of diseases, as well as of rabies, scarlet fever, trachoma, and a few others, are all tiny coccus-like forms which have the power of producing an envelope from the host cell substance, such envelope with its contained organism constituting the specific body which others have called a protozoön. Prowazek calls the group *Chlamydozoa* and says they probably stand between the bacteria and the protozoa in systematic classification. From our studies on this whole group of diseases we have come to the conclusion that there is no close relationship between the trachoma bodies and the intracellular bodies of rabies, smallpox and scarlet fever.

FIG. 193

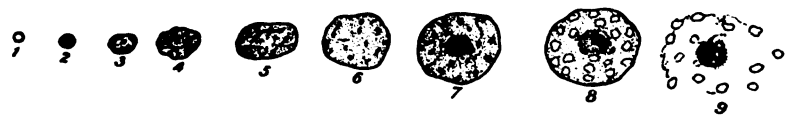


Epithelial cells of a rabbit's cornea, containing many "vaccine bodies." Tissue fixed three days after inoculation with smallpox virus. *a* and *d*, vaccine bodies; *b* and *c*, nuclei. × 1500 diameters.

In our own work on sections, which has extended irregularly over a period of several years, we have gotten results which are somewhat confusing, principally so because of the non-uniformity of the appearances of these bodies, both by different methods and by the same methods at different times. There is no doubt that, whatever the nature of the bodies, they are easily affected by methods used for fixing, hardening, and staining them. This accounts in part for the varied results reported. However, in the most perfectly prepared specimens, judged according to the appearance of the red blood cells, leukocytes, and tissue cells at a distance from the lesions, we have found that the vaccine bodies, especially in corneal infection, show a more or less constant series of changes, somewhat similar to those described by Calkins in his "gemmule formation" and by Tyzzer in his development of the vaccine bodies. This series of changes might be represented somewhat schematically as in Fig. 194.

One can easily see that such tiny bodies as these possible spores, with no definite characteristic staining qualities, would be with difficulty, if at all, differentiated from the mass of cell granules in the degenerated areas of the lesion; and, as the outline and structure of most of the other forms seem to be easily disturbed, the whole question as to their nature is, from a morphologic standpoint alone, a very difficult one to settle.

FIG. 194



Schematic representation of vaccine bodies seen within the epithelial cells in the lesions of smallpox and vaccinia: 1, spore (merozoite, sporozoite?); 2, small form which stains solidly with basic stains; 3, larger form which contains central, more darkly staining granule; 4, larger form, with more lightly staining reticular cytoplasm. This form and the next may have amœboid outline, and there may be larger amœboid forms which might be interpreted either as the grown single form or as the fusion of two or more forms; 5, form containing two central, darkly staining bodies; 6, form containing many bodies taking basic stains more or less intensely; 7, form containing a central body staining faintly with basic dyes, and small rounded bodies about it, some taking basic and some acid stains; 8, same as 7, except that many of the bodies surrounding the central body are definitely ring-shaped, and all take the acid stain. These forms vary in size; some are larger than the host nucleus; 9, form breaking up (spores set free?).

Our best results on corneas have been obtained with the following technique: Fix in Zenker's fluid for from four to eight hours; wash in running water overnight; place in 95 per cent. alcohol (changing in two hours to fresh) for twenty-four hours, then in absolute alcohol for twenty-four hours. Imbed in paraffin. The cuts should be from 3μ to 5μ thick. Stain with (1) eosin and methylene blue (Mallory)—eosin half an hour, methylene blue two minutes; (2) Heidenhain's iron hæmatoxylin; (3) Borrel modified by Calkins.

The vaccine bodies may be studied for a short sime in the living cornea by rapidly excising an inoculated cornea, spreading it on a shallow agar plate and dropping a thin cover-glass over it. The structured bodies are very clearly differentiated from the rest of the cell contents, and interesting changes have been observed in them. Too little work has been done, however, by this method, to draw any further conclusions in regard to their nature. Councilman and Tyzzer have photographed these living cornea bodies with the ultra-violet light, and the structure has come out as the chromatin structures of known living cells.

Pathogenesis.—For Lower Animals.—Various animals seem to contract the disease, or a modification of it, in nature. Horsepox, sheeppox, and cowpox, all show similar pathological changes. Experimentally, probably all mammals are susceptible though in varying degrees. Most of them are more sensitive to vaccinia than to variola. The epidermis of rabbits, for instance, shows a beautifully typical eruption after inoculation with vaccine virus, while material from smallpox eruptions produces only diffused redness. The corneal "take," however, in both instances, is similar in intensity. Monkeys are equally susceptible to both forms of the disease.

For Man.—Without vaccination human beings seem to be equally susceptible to infection with variola, whatever their race or their condition in life or in whatever part of the world they live.

The **immunity** caused by successful vaccination is not permanent, and varies in its duration in different individuals. Although it usually gives protection for several years and may give it for ten or fifteen years, it is not well to count on immunity for more than one year, and whenever one is liable to exposure it is well to be vaccinated. If this vaccination were unnecessary it will not be successful, while if it is successful we have reason to believe the individual was open at least to a mild smallpox infection.

Protective Substances Present in the Serum of Animals after Successful Vaccination.—It has been frequently shown that the blood serum of a calf some days after an extensive vaccination possesses feeble protective properties, so that the injection of one or two litres of it into a susceptible calf would prevent a successful vaccination. A further and more convincing fact has been demonstrated by Huddleston and others, namely, that when active vaccine is mixed in certain proportions with serum from an animal which had just recovered from a successful vaccination, and the mixture is inoculated into a susceptible animal, there is no reaction.

The Preparation of Vaccine.—For most of the following suggestions we are indebted to Dr. J. H. Huddleston, who has had the immediate charge of the production of vaccine for the New York Health Department for some years:

Seed Virus.—A sufficient amount of vaccine virus should be on hand to vaccinate forty to fifty persons. Five children in good health, and not previously vaccinated, should then be vaccinated, each in a spot the size of a ten-cent piece. On the fifth day after vaccination the top of the resulting vesicle should be removed and sterilized bone slips be rubbed on the base thus exposed. From one to two hundred slips on each side of the slip may be charged from each child. The slips should be allowed a moment in which to dry and then be placed in a sterilized box, in which, if kept in cold storage, they will probably remain efficient for at least two or three weeks. Rabbits are now used by us alternately with children to obtain seed virus.

Animals.—The preferable animals are female calves, from two to four months of age, in good condition and free from any skin disease. These can easily be vaccinated on the posterior abdomen and inside of the thighs by placing them on an appropriate table. It is possible that, on account of the character of the available supply, older animals may be desirable, but the calves take more typically and are more easily handled. When an animal is too old to be thrown and held without difficulty it may be vaccinated on the rump, each side of the spine; but the skin there is tougher than on the posterior abdomen and inside of the thighs, and the resulting virus, though efficient, is not so easily emulsified.

Vaccination.—The hair should be clipped from the entire body when the animal is first brought into the stable and the calf should be cleaned thoroughly, including the feet and the tail. Just before vaccination the posterior abdomen and insides of the thighs are

shaved and the skin beneath washed in succession with soap and water, sterilized water and alcohol, and then dried with a sterile towel. On this area there are now made about one hundred scarifications, each from one-quarter to one-half of an inch square. The scarification is made most easily by cross-hatching with a six-bladed instrument the blades being about one-thirtieth of an inch apart. The scarification is superficial, but brings a small amount of blood. An area as small as specified is less likely to become infected than a larger one. The scarifications should be separated from each other by an interval of at least one-half to three-quarters of an inch. After they have been made they should be dried with a sterile towel or with sterile cotton and rubbed with the charged slips. One to two slips, depending on the amount of virus each slip holds, should be sufficient for vaccinating each vesicle.

Collection.—On the fifth or sixth day, depending upon the rate of development of the vaccine vesicles, they should be ready for collection. The entire shaved area is washed with sterile water and sterile cotton, and the crusts are picked off. The soft, pulpy mass remaining is then curetted off with an ordinary steel curette and the pulp placed in a sterilized vessel. After the curettage, serum exudes from the torn base of the vesicle, and ivory slips may be charged in this. The pulp should be mixed with four times its weight of glycerin and water (50 per cent. glycerin, 49 per cent. water, 1 per cent. carbolic acid), and this is done most effectively by passing the mixture between the rollers of a Doring mill. The more watery the pulp, especially if it is not to be used immediately, the smaller should be the proportion of glycerin. The emulsion so produced can then be put up for issue in vials. The slips charged with the serum from the calf may also be used for vaccinating. Capillary tubes require especial means of filling, and small vials filled and corked answer the purpose admirably.

Propagation.—Subsequent animals may be vaccinated in any one of the three ways: (*a*) slips may be charged from typical vesicles on primary vaccinations, just as with the first calf, and used for seed virus; (*b*) slips charged with the serum from the calf may be used to vaccinate a second calf; (*c*) the glycerinated emulsion may be used to vaccinate succeeding calves, but in the last case it is necessary to keep the emulsion a varying length of time—often two or three months—before it is fit for use in vaccination of the calf, since the employment of fresh glycerinated pulp on a succession of calves leads to prompt degeneration of the vaccine and to the production of infected vesicles.

Care of the Calves.—All bedding is avoided and an exclusively milk diet given; thus much of the otherwise unavoidable dust is done away with.

Laboratory.—The laboratory should consist of at least three rooms: (*a*) stable; (*b*) operating-room; (*c*) laboratory-room. It should be possible to make and keep all the rooms clean. The stable and operating-room should be flushed with a hose and hot water daily. Excreta should be removed immediately. The calves can be kept clean

if they stand on a raised and perforated platform which is so short that the defecations cannot fall on it and if they have no bedding. They must be fastened to keep them from kicking the scarifications. In the health department, when a calf is removed, its stall and platform are scoured with a brush and sodium carbonate solution. The stable should be provided with a shovel, broom, hose, horse clipper, cord, and with halters, buckets, scrubbing brushes, and sponges. The operating-room should be well-lighted and provided with a table and with stools.

The only requisites for the table are that it should be heavy and firm; that it should have holes through the top so arranged that straps can be pasesd through them to hold the calf down, and a vertical strip on one side of the table to which the upper hind leg of the calf can be fastened. The calf can be thrown upon the table easily by two attendants.

The laboratory should also be well-lighted and furnished with tables, chairs, desk, case for instruments, and refrigerator. It should also have both a steam and a dry-air sterilizer, a set of scales weighing to grams or centigrams, and a blast lamp and bellows. In stock there should be one to two thousand bone slips for seed virus and ten to fifteen thousand smaller slips for issue; two or more scarifiers; a curette; four to six razors for shaving the animals; a razor strop; a pair of large scissors, curved on the flat, for clipping the animals; a burette, from which glycerin flows while the vaccine pulp is being ground; a burette holder; a Doring vaccine grinder; clinical thermometers to take the temperature of the animals; six to twelve small glass dishes with covers; a hard-rubber syringe, of four-ounce capacity, to make suction; absorbent cotton; glass vials and corks; and several pounds of soft glass tubing, three-eighths of an inch in calibre, to store virus emulsion. There should also be gowns and caps for the attendants. Sodium carbonate, bichloride of mercury, bromine for a deodorizer, alcohol, carbolic acid, and glycerin are the chemicals needed.

For issue for public vaccinations there are also needed packing-boxes, rubber bands, sheet wadding, needles, and wooden tooth-picks for removing the virus from the vials and rubbing it on the sacrifications.

Yield.—The material obtained from the five children should vaccinate at least five calves; it may easily vaccinate fifteen calves. Ten grams of pulp and two hundred charged slips would be an average yield from a calf, and that, when made up, should suffice to vaccinate at least fifteen hundred persons. Calves vary immensely in the yield. Of two calves vaccinated in precisely the same way one may furnish material for five hundred vaccinations and the other for ten thousand vaccinations.

The Durability of Glycerinated Virus in Sealed Tubes.—As a result of testing from time to time an immense number of specimens of vaccine, the conclusion has been reached that vaccine properly put up should keep at least three months. From time to time a single

lot of virus will fail by the end of one month. Sometimes this is due to the glycerin, as when it has some chemical impurity or it is not diluted sufficiently. When kept below the freezing point it holds its activity for a longer time.

Bacteria in Vaccine.—It is impossible to prepare vaccine on a large scale so that it is at the time of its removal free from bacteria. In fact, there are usually very large numbers of one or more varieties of bacteria present. When the stable and animals have been kept clean the bacteria comprise usually very few varieties; when dirty conditions prevail the bacterial varieties are more numerous. The number of bacteria found varies enormously. The largest number found by us in vaccine pulp from the calf was 126,360 in one loopful, and the smallest number 523. Discrete vesicles at the borders contain many less bacteria than the confluent ones caused by the inoculation at the scarification. The pulp has many more bacteria than the serum of the vesicles. The period which elapses before glycerinated virus becomes sterile is also quite variable, but does not depend in any direct way upon the number of bacteria originally present. A very large number may disappear rapidly, and a few persist.

After two or three weeks the number of living bacteria is usually greatly diminished, especially after addition of glycerin-carbolic mixture, when they entirely disapppear. Pathogenic bacteria other than the practically non-virulent skin staphylococci are not found when animals are properly kept and vaccinated.

Rabbit Vaccine.—Upon rabbits a practically bacteria-free vaccine can be obtained, and many laboratories now use rabbits not only to intensify the virus, but to free it from bacteria. (See p. 613 for method of obtaining vaccine from rabbits.)

Inoculation of Human Beings.—Efficient vaccine should be inoculated in a portion of skin no more than one-sixteenth inch in diameter.

SCARLET FEVER.

Scarlet fever is an acute febrile, highly infectious disease, characterized by a diffuse punctate erythematous skin eruption, accompanied by catarrhal, croupous, or gangrenous inflammation of the upper respiratory tract and by manifestations of general systemic infection.

Historic Note.—The disease was probably known long before the Christian era, but the present name does not appear until the time of Sydenham (1685), who differentiated the disease from measles. The cause is still undetermined.

Occurrence.—It is very generally disseminated, but is much more common in temperate climates than in the tropics.

Etiology.—The specific exciting factor is thought by many to be a streptococcus, of the *Streptococcus pyogenes* type, but the evidence in favor of this view is very slight.

Recently Mallory reported the presence in scarlet fever of certain bodies which he considered protozoa and the probable cause of the

disease. He summarized his observations as follows: "In 4 cases of scarlet fever certain bodies were found which in their morphology strongly suggest that they may be various stages in the developmental cycle of a protozoön. They occur in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium. The great majority of the bodies vary from 2μ to 7μ in diameter, and stain delicately but sharply with methylene blue. They form a series of bodies, including the formation of definite rosettes with numerous segments, which are closely analogous to the series seen in the asexual development (schizogony) of the malarial parasites, but in addition there are certain coarsely reticulated forms which may represent stages in sporogony or be due to degeneration of the other forms." He has given the name *Cyclasterion scarlatinale* to these bodies in consequence of the frequent wheel and star shapes of the rosettes. In our laboratory Field in 1905 examined 10 scarlet fever autopsies and 20 specimens of living skin taken from patients at different stages of the disease, together with a number of control specimens taken from measles, antitoxin rashes, and diptheria; but he was only able to find a few of Mallory's less characteristic forms, and these only in the scarlet fever autopsy cases.

Duval (1904) made the announcement that in fluid obtained through blistering the skin of scarlet fever patients by a very quick method he has obtained bodies which he interprets as forms of Mallory's protozoön.

Field obtained similar bodies by the same method in both scarlet fever and measles cases, and in four cases of scarlatiniform antitoxin rashes, more in the first two groups than in the last. He obtained them in no other cases so far examined. Field came to the conclusion that the majority of them are from degenerated leukocytes.

Since 1905 we have continued the studies on the etiology of scarlet fever, both from the protozoan and the bacteriologic standpoints. We have examined the skin of 46 new cases (17 living) and other organs from 5 autopsies, and though we have found interesting bodies in the tissue taken from the *living*, as well as from the dead, some corresponding to Mallory's less definite forms, we have been unable to demonstrate morphologic characteristics distinct enough to place these bodies among the microörganisms. (Plate VIII, Fig. 1.)

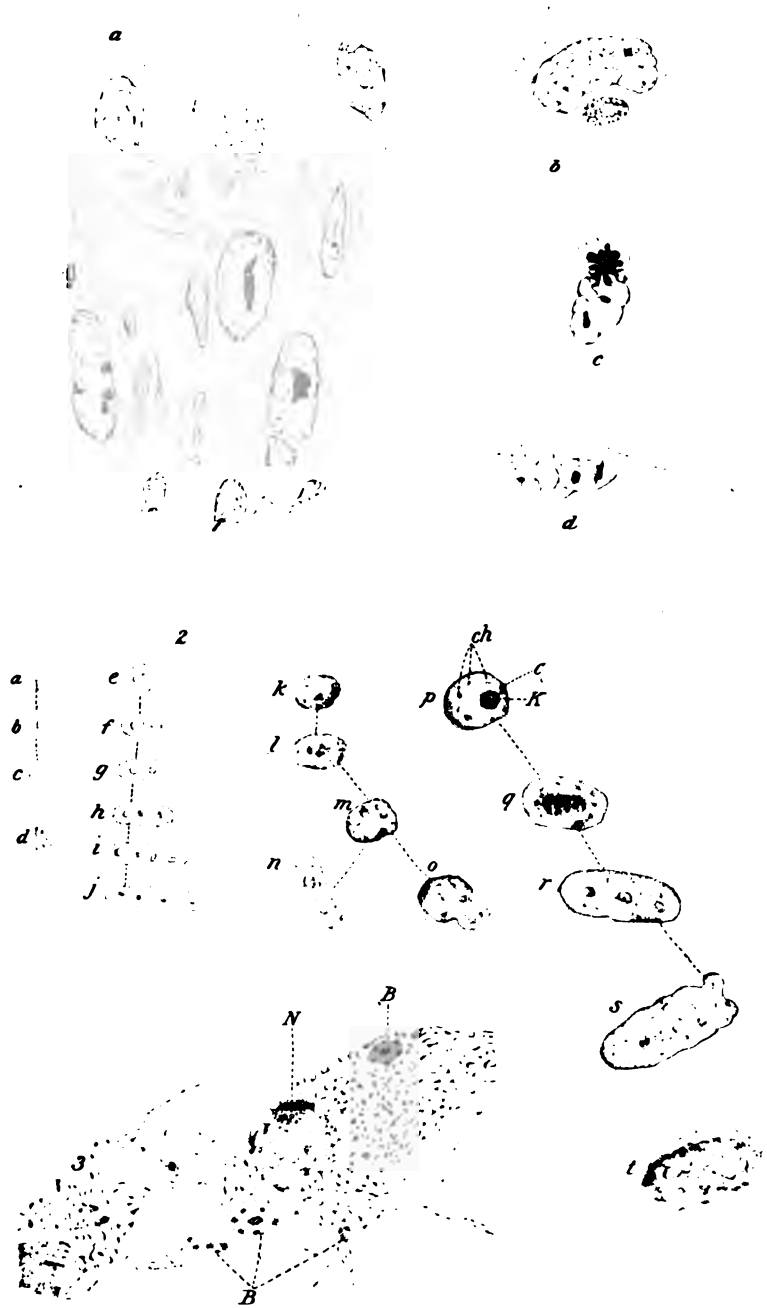
MEASLES.

Field states that he found a moderate number of delicately staining nucleate bodies in the skin and blister fluid of measles as well as in scarlet fever, but does not suggest their nature.

A tiny influenza-like bacillus has been found by several observers in the blood and nasal discharges of measles cases, but nothing has been proved in regard to its causal relationship to the disease.

Hektoen (1905) produced measles in two human cases by the inoculation of the blood drawn from an infected case at an early stage of the disease.

PLATE VIII



1. Those of Mallory's scarlet fever bodies, similar to the ones found by us: *a*, in corium; *b*, *c*, *d*, "division stages" within epithelial cells (eosin and methylene blue). 2. Various division forms of the negri bodies (Giemsa stain). For description see text. 3. Smear of Ammon's horn of dog, showing negri bodies (*B*) stained red in the large blue-stained nerve cells; *N*, nucleus of nerve cell. (Van Gieson's fuchsin and methylene-blue stain.)

Ewing reports the finding of peculiar granules or ring-shaped structures in apparent vacuoles about epithelial nuclei and in capillaries and lymph spaces of the skin. These were in large numbers in a case of hemorrhagic measles and in smaller numbers in other cases. Ewing thinks that the most probable hypothesis in regard to their nature is that they represent a coagulated albuminous material derived from the blood and from degenerating epithelium. We have also found large numbers of similar bodies in a fatal case of acute measles, and fewer forms in less severe cases.

TRACHOMA.

A good deal of work has been done recently on the etiology of this eye disease, which is a progressive follicular inflammation of the conjunctivæ followed by cicatrization. Prowazek in 1907 announced that the cause of the disease is a tiny organism which grows in a characteristic manner in the conjunctival epithelial cells. The organism itself he says is very small, so small that at first it cannot be seen, only the mantle which it produces is demonstrable; this stains blue with Giemsa, and as the organisms grow in bunches, one sees at first in the neighborhood of the nucleus only a bunch of tiny blue coccus-like bodies. The organism finally appears as a tiny red granule within the blue body. As it continues to increase in numbers and size the blue mantles finally disappear, leaving a mass of small rounded or slightly elongate red bodies. The bodies are only found in the early acute cases. Prowazek named them *Chlamydozoa* on account of their mantle, and thinks they should occupy a place between bacteria and protozoa (see also pp. 488 and 623).

We have examined about 260 cases, chiefly school children, diagnosed clinically trachoma; and, while we have found "trachoma bodies" in many (14) of the early acute cases (23), the others have shown nothing, thus indicating (if these bodies are diagnostic) either that the great majority of our school children have not true trachoma or that the "bodies" are too few in these chronic cases to be of practical aid in diagnosis.

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CHAPTER XLVII.

RABIES. YELLOW FEVER.

RABIES.

Introduction.—Rabies (synonyms: Hydrophobia, Lyssa, Hundswuth, Rage) is an acute infectious disease of mammals, dependent upon a specific virus, and communicated to susceptible animals by the saliva of an infected animal coming in contact with a broken surface, usually through a bite. The name rabies is given to the disease because of its most frequent and characteristic symptom—furor or madness. Hydrophobia (Greek, fear of water) is another name commonly used, which is also given because of a frequent symptom of the disease, the apparent fear of water. Lyssa is a Greek word indicating still another symptom, *i.e.*, swollen follicles on the under surface of the tongue. Within the gray nerve tissue of rabid animals are peculiar protozoön-like structures known as "Negri bodies" which are diagnostic of rabies. The nature of these bodies is still a question of dispute.

Historical Notes.—Rabies is probably one of the oldest diseases in existence, but because of the occurrence of so few human cases, and because the disease develops so long after the bite, its source was for a long time not known nor was it recognized as a separate disease. Hippocrates does not mention it in his writing, but Aristotle about 50 years later (about 300 B. C.) speaks of its being purely an animal disease and being carried by the bite of one animal to another. Celsus in the first century was the first to give in writing a detailed description of human rabies. He speaks of it being produced by the bite of rabid animals and states that the wound must be thoroughly bathed and then burned with a hot iron in order to prevent the development of the disease, for after symptoms appear death always follows. As Celsus was not a physician he must have gotten his knowledge from writings which have since been lost. Other writers soon after gave very true descriptions of the symptoms and handling of the disease.

Many hundred years passed after this without adding anything to our knowledge of the disease, though authors on the subject were numerous. Van Sweiten in 1770 observed the paralytic form of rabies in human beings. At this time several authors, among them Morgagni and Zwinger, believed that the bite of a dog which was not suffering from rabies might produce the disease in man. In 1802 Bosquillon brought forth the original idea that belief in the existence of infectious material in rabies was a chimera and that hydrophobia was simply due to fright. This false idea had adherants for a long time; even now, by a few people, it is throught to be a true one.

Among the host of good observers who studied the disease during the latter part of the nineteenth century, Pasteur stands out as the discoverer, in 1880, of the fact that the disease may be prevented by inoculating gradually increasing doses of the virus into the person or animal bitten. This treatment with some modifications, the details of which will be given later, is still used, though many efforts have been made to develop an efficient serum treatment. Pasteur, as well as numerous other investigators, tried to dis-

cover the specific cause of rabies, but all of their results were negative. The importance of making a quick diagnosis had become so evident that the efforts of many workers were directed toward this end alone.

Pasteur and his immediate followers relied for their diagnosis entirely upon rabbit inoculations, and this meant a fifteen to twenty days' wait before the patient knew whether or not the treatment he was receiving was necessary. In 1898 this time was shortened to about nine days in our laboratory by Wilson, who found that guinea-pigs came down with the disease much more quickly than rabbits. From time to time it has been thought that certain histologic findings were diagnostic; for instance, the "rabic tubercles" of Babes, and the areas of "round and oval-celled accumulation in the cerebro-spinal and sympathetic ganglia" of Van Gehuchten and Nelis, were said to be specific, but further study has shown that they are not absolutely specific for rabies. In many cases the whole picture of the grosser histologic changes is sufficiently characteristic to warrant the diagnosis of rabies, but often it is not so.

It is not until Negri, in 1903, described certain bodies (Negri bodies) seen by him in large nerve cells in sections of the central nervous system, that anything was found which seemed absolutely specific for hydrophobia. Negri claims that these bodies are not only specific for rabies, but that they are probably animal parasites and the cause of the disease.

Negri's later studies confirm his previous work and add some new facts in regard to the structure of the larger bodies.

His work, especially as far as the diagnostic value of these bodies is concerned, has been corroborated by investigators in almost all parts of the scientific world, among them workers in our own laboratory who not only determined their worth in diagnosis, but investigated their nature.

In our work emphasis was placed upon the fact that the demonstration of the "Negri bodies" by our "smear method" (see p. 624) wonderfully simplified the process of diagnosis. As a result of our studies we concluded that the Negri bodies are not only specific for rabies, but that they are living organisms, belonging to the protozoa, and are the cause of the disease; giving as our reasons the following facts: (*a*) They have a definite, characteristic morphology; (*b*) This morphology is constantly cyclic, that is, a definite series of forms indicating growth and multiplication can be demonstrated; (*c*) The structure and staining qualities, as shown especially by the smear method of examination, resemble those of certain known protozoa, notably of members of the rhizopoda.

Since this report was published many more cases of rabies have been more or less studied by us and our former conclusions have been more firmly established. Indeed, the evidence as to animal nature of these cell inclusions seemed so convincing that Williams in 1906 gave them the name *Neuroryctes hydrophobiæ.*[1] Calkins has since studied these bodies and agrees with Williams as to their nature. He called attention to the similarity between their structure and that of the rhizopoda.

A number of observers, however, still believe that the Negri body as a whole is principally the result of cell degeneration and that the specific organism may be contained within it. Prowazek includes rabies with his "chlamydozoan diseases" (see p. 621). To anyone who has studied the two diseases, however, there can be no question in regard to the essential difference between the "trachoma bodies" and the "Negri bodies."

[1] Proceedings of the N. Y. Pathological Society, 1906, VI, 77.

Material and Methods for Study.—In New York one may almost constantly obtain fresh brains of rabid animals, from veterinary hospitals or from the laboratories handling this material. Two methods have been used in helping to study the principal site of infection.

(1) Animal inoculations; (2) Sections and smears.

The first method is used as a decisive test in diagnosis when results from the second method are doubtful.

The technic of the *smear method* used at present in the Research Laboratory of the New York City Health Department is as follows:

1. Glass slides and cover-glasses are washed thoroughly with soap and water, then heated in the flame to get rid of oily substances.

2. A small bit of the gray substance of brain chosen for examination is cut out with a small sharp pair of scissors and placed about one inch from the end of the slide, so as to leave enough room for a label. The cut in the brain should be made at right angles to its surface and a thin slice taken, avoiding the white matter as much as possible.

3. A cover-slip placed over the piece of tissue is pressed upon it until it is spread out in a moderately thin layer; then the cover-slip is moved slowly and evenly over the slide to the end opposite the label. Only slight pressure should be used in making the smear, but slightly more should be exerted on the cover-glass toward the label side of the slide, thus allowing more of the nerve tissue to be carried farther down the smear and producing more well-spread nerve cells. If any thick places are left at the edge of the smear, one or two of them may be spread out toward the side of the slide with the edge of the cover-glass.

4. For diagnosis work such a smear should be made from at least three different parts of gray matter of the central nervous system: first, from the cortex in the region of the fissure of Rolando or in the region corresponding to it (in the dog, the convolution around the crucial sulcus); second, from Ammon's horn, and, third, from the gray matter of the cerebellum.

5. The smears are partially dried in air and fixed for about ten seconds in *neutralized* methyl, alcohol to which one-tenth per cent. picric acid is added

6. The excess of alcohol is removed by pressing fine filter-paper gently over the smear.

7. The methylene-blue-fuchsin staining mixture recommended by Van Gieson is poured over the slide, warmed until it steams, poured off, and the smear is washed in running tap water, and allowed to dry, the excess of water being removed with fine filter-paper.

The staining mixture recommended by Van Gieson is made by us at present in the following proportions:

5 c.c. distilled H_2O; 10 drops sat. alc. sol. meth. blue; 2 drops sat. alc. sol. basic fuschsin.

This mixture in room temperature in diffuse daylight will keep for a day, and possibly two. In the dark at room temperature it retains its staining powers a little longer. At ice-box temperature it lasts a much longer time, probably indefinitely.

With this method the Negri bodies stain magenta, their contained granules blue, the nerve cells blue, and the red blood cells yellow.

Other methods we have found useful for staining smears are the following:

Giemsa's Solution.—Smears are fixed in neutralized methyl alcohol for one minute. The staining solution recommended last by Giemsa[1] (1 drop of

[1] Azur II—Eosin ..... 3.0 g.
Azur II 0.8
Glycerin (Merk. chem. pure) ..... 250.0 c.c.
Methyl alcohol (chem. pure) 250.0

Both glycerin and alcohol are heated to 60° C. The dyes are put into the alcohol and the glycerin is added slowly, stirring. The mixture is allowed to stand at room temperature overnight, and after filtration is ready for use.

The solution is prepared ready for use by Grübler, Leipzig.

the stain to every c.c. of distilled water made alkaline by the previous addition of one drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of the water) is poured over the slide and allowed to stand for one-half to three hours. The longer time brings out the structure better, and in twenty-four hours well-made smears are not overstained. After the stain is poured off, the smear is washed in running tap water for one to three minutes, and dried with filter-paper. If the smear is thick, the "bodies" may come out a little more clearly by dipping in 50 per cent. methyl alcohol before washing in water; then the washing need not be as thorough. By this method of staining, the cytoplasm of the "bodies" stains blue and the central bodies and chromatoid granules stain a blue-red or azur. Generally the larger "bodies" are a darker blue than the smaller, the smallest of all may be very light. The cytoplasm of the nerve cells stains blue also, but with a successfully made smear the cytoplasm is so spread out that the outline and structure of most of the "bodies" are seen distinctly within it. The nuclei of the nerve cells are stained red with the azur, the nucleoli a dull blue, the red blood cells a pink-yellow, more pink if the decolorization is used. The "bodies" have an appearance of depth, due to their slightly refractive qualities.

The Eosin-methylene-blue Method Recommended by Mallory.—The smears are fixed in Zenker's solution[1] for one-half hour; after being rinsed in tap water they are placed successively in 95 per cent. alcohol + iodine one-quarter hour, 95 per cent. alcohol one-half hour, absolute alcohol one-half hour, eosin solution twenty minutes, rinsed in tap water, methylene-blue solution fifteen minutes, differentiated in 95 per cent. alcohol from one to five minutes, and dried with filter-paper. With this method of staining, the cytoplasm of the "bodies" is a magenta, light in the small bodies, darker in the larger; the central bodies and chromatoid granules are a very dark blue, the nerve-cell cytoplasm a light blue, the nucleus a darker blue, and the blood cells a brilliant eosin-pink. With more decolorization in the alcohol the "bodies" are not such a deep magenta, and the difference in color between them and the red blood cells is not so marked.

The technique of the *section* work is as follows: (1) The small pieces are left in Zenker's fluid for three to four hours; (2) washed in tap water for five minutes; (3) placed in 80 per cent. alcohol + iodine[2] (enough tincture of iodine added to give port-wine color) for about twenty-four hours; (4) 95 per cent. alcohol + iodine twenty-four hours; (5) 95 per cent. alcohol twenty-four hours; (6) absolute alcohol from four to six hours; (7) cedar oil until cleared; (8) cedar oil + paraffin 52° two hours; (9) paraffin 52° two hours in each of two baths; (10) boxing; (11) sections are cut at 3 to 6μ, dried in thermostat at 36° C. for about twenty-four hours protected from the dust, and stained according to the eosin and methylene-blue method recommended by Mallory. The most important point in the technique is the time the material is allowed to remain in Zenker. According to our experience, two hours' fixation is not enough, three to eight hours is very good, and with every hour after eight hours the results become less satisfactory. Left in Zenker overnight the tissue is granular and takes the eosin stain more or less deeply, both of which results interfere with the appearance of the tiniest "bodies," especially of the very delicate, tiny forms found by us in sections from fixed virus.

In regard to the rest of the technique, it is sufficient to say that the changes to the different fluids are made with great regularity, and the final differentiation in alcohol of the stained sections is done most carefully.

In the sections made in this way we have been able to demonstrate clearly very tiny forms, as well as good structures in the larger forms.

[1] See p. 522.

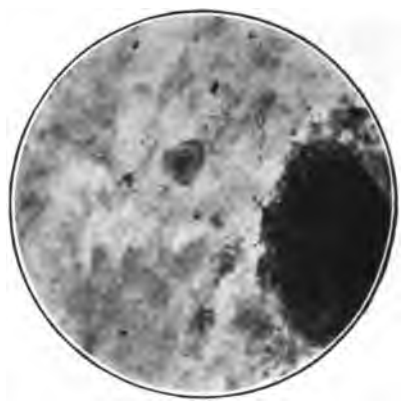
[2] Better results are obtained by treating the tissue with iodine after the sections are cut, just before staining, as they then do not need to be so long a time in the iodine solution—ten minutes to half an hour being sufficient.

Harris has recently published a new staining method for both sections and smears (see bibliography), which brings the larger bodies out clearly, but which does not seem to give enough differentiation between the smaller bodies and the nucleoli of the nerve cells.

Morphology of the Negri Bodies (Plate VIII, Figs. 2 and 3).—The largest forms measured are about 18μ and the smallest about 0.5μ. They are round, oval, oblong, triangular, or amœboid. The latter are more numerous in the fixed virus of rabbits and guinea-pigs. Their structure is shown especially well in smears. Whatever the variety or species of animal infected, the bodies present the same general characteristic structure; *i. e.*, a hyaline-like cytoplasm with an entire margin, containing one or more chromatin bodies having a more or less complicated and regular arrangement.

Their structure varies to a certain extent with their size. In fixed virus, with an occasional exception, only tiny forms are found (Plate VIII, Fig. 2 *a–d*). These are rounded or sometimes wavy in outline.

FIG. 195



Negri body showing central chromatin with ring of small granules. × 2000.

as if possessing slight amœboid motion, sometimes elongated, extending along the rim of the host-cell nucleus, or along one of the nerve fibrils, as if moving there; with eosin and methylene blue they take a delicate light magenta stain, very similar to that taken by the small serum globules in the blood vessels. Many of the organisms, however, show a small chromatin granule, situated more or less eccentrically, sometimes on the very rim of the body. In the larger forms the granule is large, in the smaller it cannot always be seen; some of the larger forms show from two to several granules and occasionally there is a body with the definite central body and the small granules about it.

Detailed Characteristics of Structure in the Large Forms (Fig. 195 and Plate VIII, Fig. 2 *e–f*).—In smears, as well as in sections, the *cytoplasm* appears quite homogeneous; there is no evidence of a reticulum or of a granular structure outside of the definite chromatoid granules.

The smears, however, have brought out one important point in regard to the cytoplasm more clearly than the sections, and that is that it is more basophilic than acidophilic in staining qualities. With the Giemsa stain, as we have already seen, it takes the methylene-blue stain more than the eosin-red, and even with the simple eosin methylene-blue stain the protoplasm appears as a deep magenta unless much decolorized.

In studying the *central bodies* of these organisms, as they appear in the smears, one of the first things noticeable is that they are not surrounded by a clear space—that there is no sign of a vacuolar appearance in the body. This is a very different appearance from that given in the sections, and it shows that the vacuoles seen in the sections are artefacts due to the technique. We notice next that in the great majority of the organisms the central body stands out clearly, as decidedly different in structure, and slightly so in staining qualities, from the chromatoid granules which surround it. The general type of the structure of the central body is that of many well-known protozoan nuclei.

The chromatin is arranged in a more or less granular ring around the periphery of the central body or nucleus, leaving an achromatic or more acid-staining centre in which is situated, generally eccentrically a varying-sized karyosome (Plate VIII, Fig. 2*p*). There are a number of variations from this principal type, according to stage of development. Often the whole nucleus answers to the description of the compound karyosome given by Calkins in his description of the protozoan nucleus. In the tiny "bodies," as we have said, the chromatin can only be seen as a dot; in those a little larger it may be a large solidly staining granule, or a ring or rod, the latter often hour-glass-shaped. In forms large enough for the characteristic structure to be developed and to be clearly seen, the central body may show evidence of fragmentation (Plate VIII, Fig. 1*q*, etc.).

Just such evidence of fragmentation is shown in many protozoan nuclei preparatory to division, notably among the rhizopoda. It is interesting that forms showing this phase, and, moreover, very similar in general appearance to some of the forms seen here, have been depicted by Doflein in the *early stages* of the life-cycle of *Nosema lophii*, a myxosporidium, parasitic in the ganglion cells of a fish *Lophius piscatorius*.

The fragmented particles seem to be leaving the nucleus in certain forms, and in this way presumably the chromatoid granules are produced, thus forming chromidia.

The *chromatoid granules* are most frequently arranged in a more or less complete circle about the nucleus. They are somewhat irregular in outline and size, being occasionally ring-shaped, sometimes elongated, often in two's, due probably to active changes of growth and division. They take generally a more mixed chromatin stain than the chromatin of the nucleus.

Evidences of Division.—All stages in transverse division are seen. Many evidences of budding are also seen. The chromatoid granules

divide and pass out with part of the cytoplasm as a bud. This budding or unequal division appears to take place very early in the growth of the organism and to continue throughout growth until the parent body forms a mass of small organisms which may then break apart at the same time. The budding accounts for the number of small and large forms in a single cell.

Number.—They vary in number according to the stage of the disease and to the infectivity of the part.

Site.—They are situated chiefly in the cytoplasm and along the fibres in the branches of the large nerve cells of the central nervous system. In parts of smears which are more broken up the bodies may appear as if lying free, and it is these bodies, if the pressure be not too great in smearing, that show the structure best. In some cases the bodies are distinctly localized in small scattered areas of the central nervous system. We have always found bodies in the spinal cord in abundance, but here they are especially prone to be localized in discrete groups of cells.

That the organisms are present in various glands of the body (salivary thyroid, suprarenal capsule, etc.) is shown by the virulence of emulsions from these organs. Cows' milk (Westbrook, McDaniel) and blood (Marie) have also been shown to be slightly virulent.

Diagnosis of Rabies.—In our laboratory, for the past five years, or since we have used the smear method in routine diagnosis, there have been about fifteen hundred cases in all examined, including suspected rabies and controls.

These are divided into two groups, the first comprising the cases sent in from outside, for diagnosis only, and the second, the experimental cases.

Since the publication of our work in May, 1906, in our routine work we have considered the presence of the Negri bodies in smears as diagnostic of rabies and have made no further tests except in those cases which we have used in our experimental work. Through this experimental work, however, we have added three hundred cases to the list of those which had the comparative tests, and our former conclusions have been more firmly established.

In all of our work controlled by careful animal inoculations we have never yet failed to have typical rabies develop in animals inoculated with material showing definitely structured Negri bodies. Negative results after inoculation with such material must be interpreted at present as due to some error in technique, such as regurgitation, or hemorrhage at the time of inoculation, emulsion improperly made, not enough of the virulent material taken because of localization of the organisms, etc.

Possibly individual resistance of the animal inoculated might play a part. We have used principally guinea-pigs, and some of them have shown enough irregularity in regard to the time in which they have come down with the disease to suggest a varied individual susceptibility, if other factors can be ruled out.

On the other hand, material in which we have failed to demonstrate typically structured bodies has produced rabies. All of this material, however, since we have improved our technique, has shown suspicious small forms similar to those found in rabbit-fixed virus. But any decomposing brain may also show in smears bodies very similar to these tiny forms, therefore it is difficult to rule out rabies in such cases. Of course the animal test will probably always have to be used with brains that are too decomposed to show any formed elements except bacteria, unless a reliable chemical test can be discovered.

So far we have not had rabies produced by fresh brains showing no Negri bodies and no suspicious forms, but a few observers have claimed that such material has produced the disease. Therefore, until we can standardize our technique, we must in all such cases use animal inoculations. We may, however, be reasonably certain that a case showing such negative material was not a case of rabies. We may summarize our knowledge in regard to the worth of the smear method in diagnosis as follows:

1. Negri bodies demonstrated, diagnosis rabies.
2. Negri bodies not demonstrated in fresh brains, very probably not rabies.
3. Negri bodies not demonstrated in decomposing brains, uncertain.
4. Suspicious bodies in fresh brains, probably rabies.

The *localization* of the Negri bodies is an important point in making diagnoses. We have found well-developed bodies distinctly localized in different parts of the brain in several instances. In one horse there were small widely scattered areas of well-structured forms throughout the cerebellum, while tiny indefinite forms were scattered through the rest of the brain examined. In two human brains well-developed forms were found in the corpus striatum and not in the rest of the brain. In several dogs the localization has also been marked.

The Complement Binding Test in Rabies.—This test has been tried by Heller (1907), Friedberger (1907), and Baroni (1908), with negative results. Berry (1910), in our research laboratory went over this work thoroughly and obtained similar negative results.

Effect of Chemic and Physic Agents on Rabic Virus.—Rabic virus appears to become attenuated under certain conditions of temperature; indeed, if it be subjected for about an hour to 50° C. or for half an hour to 60° C., its activity is completely destroyed. A 5 per cent. solution of carbolic acid, acting for the same period, exerts a similar effect, as do likewise 1 : 1000 solutions of bichloride of mercury, acetic acid, or potassium permanganate. The virus also rapidly loses its strength by exposure to air, especially in sunlight; when, however, protected from heat, light, and air it retains its virulence for a long period.

Pathogenesis.—Natural Infection.—The disease occurs in nature among the following animals given in order of their frequency: dogs, cats, wolves, horses, cows, pigs, skunks, deer, and man; in fact, as all

warm-blooded animals are more or less susceptible to inoculations, all may presumbly contract the disease when an open wound is brought in contact with infectious material of a rabid animal.

Rabies occurs in almost all parts of the world. It is most common in Russia, France, Belgium, and Italy; it is not infrequent in Austria and in those parts of Germany bordering on Russia. In this hemisphere it is infrequent in Canada, but in the United States the cases are increasing in numbers, especially during the last year when there have been several epidemics in some of the northwestern States. In California several cases have recently been reported. In England, Mexico, and South America it occurs occasionally; while in England, North Germany, Switzerland, Holland, and Denmark, because of the enforced quarantine laws, and to the wise provision that all dogs shall be muzzled, it is extremely rare. In Australia it is unknown, probably because the law that every dog imported into the island must first undergo a six months' quarantine has always been enforced. In the vicinity of New York the disease seems to be on the increase.

The *contagion* is supposed always to be carried through the bite of a rabid animal or through the sputum of such an animal coming in contact with an open wound.

In this connection the question as to how long the sputum of a rabid dog may remain virulent after it drops from the animal is an interesting one. A case came under our observation in 1906 which illustrates this point. A child of six years came down with typical rabies in a neighborhood where there had recently been several cases of canine rabies, but no history of a bite could be obtained. The parents were sure she had not been bitten. Six weeks before, however, the child had fallen in the street and cut her cheek severely on a jagged stone. The wound was cauterized and healed without further trouble. A mad dog had been on that street just before this occurred. It is reasonable to suppose that the stone had on it some of the sputum from that dog, and so the child was infected. Such a case would not occur very often, but the possibility should be considered.

In regard to the question as to whether the bite of apparently healthy animals may give the disease, it may be said that, judging from laboratory experiments, some animals may have a light attack of the disease and recover spontaneously, though such cases, if they occur, are probably extremely rare. That the bite of an infected animal may give the disease before that animal shows symptoms has been proved. Nine days is the longest time reported between a bite and the appearance of symptoms. Therefore, if an animal is kept under observation two weeks after biting another, without developing symptoms, he may be pronounced free from suspicion.

Occupation seems to have an effect upon the number of cases among humans in one way. Those people who are much in the country or on the streets—in other words, those who might come most frequently in contact with rabid animals—most frequently contract the disease; otherwise neither age, sex, nor occupation has any effect.

The time of the year seems to have little effect, though most cases

are said to occur during the summer months. The numbers vary with different years. In 1907 for instance we had as many cases in January as in August and in September and more in June than in any other month.

The certainty with which the disease may be produced after a bite and the rapidity of its development have been found to be governed by three factors: (1) the quantity of the rabic virus introduced; (2) the point of inoculation; (3) the strength of the virus as determined by the kind of animal which affords the cultivation ground for the growth of the organism. It is a matter of common observation in man that slight wounds of the skin of the limbs and of the back or wherever the skin is thick and the nerves few either produce no results, especially when bites are made through clothes, or are followed by the disease after an extremely long period of incubation; while in lacerated wounds of the tip of the fingers where small nerves are numerous or where the muscles and nerve trunks are reached, or in lacerated wounds of the face where there is also an abundance of nerves the period of incubation is usually much shorter and the disease generally more rapid.

These facts explain why only about 16 per cent. of human beings bitten by rabid animals and untreated appear to contract hydrophobia.

Since the establishment of the Pasteur treatment for the disease, the percentage of developed cases after bites is very much less, a fraction of 1 per cent.

Symptoms.—There is always a decided incubation period after the bite which varies within quite wide limits, but in the great majority of cases it is from twenty to sixty days. Any period after six months is an exception; the shortest we have on record is fourteen days and the longest authentic period is seven months. A very few apparently authentic cases have been reported as developing in about one year, but reports of any time beyond this must be received with doubt. After treatment, however, a few cases have been reported as occurring later than this, but even here the question of reinfection is not absolutely ruled out. We had a case illustrating this: One of our patients, a man who helped a dog veterinarian, was treated after a severe wound from a rabid animal, and fourteen months later came down with typical hydrophobia; but we found that since his treatment he had become very careless with cases of rabies because he considered himself immune. He was warned that there might be danger, but six weeks before his death he put a wounded hand into the mouth of a rabid animal. There seems to be no doubt but that it was a case of reinfection after loss of protection from the treatment rather than one of delayed rabies.

The wound heals as other wounds and sometimes shows no further symptoms. Occasionally, however, redness and swelling of the scar have been reported; oftener there are pains extending from the scar along the nerve paths to the brain.

The symptoms may be divided into three stages. First, the prodromal or melancholic stage; second, the excited or convulsive stage; and, third the paralytic stage.

When the second stage is the most pronounced the disease is called furious or convulsive rabies; when this stage is very short or practically lacking and paralysis begins early, the disease is called dumb or paralytic rabies.

In the dog rabies appears in the two typical forms, the furious and the

paralytic. The principal symptoms of each form may be summarized as follows: (*a*) *Furious rabies;* change of behavior, biting (especially at those to whom the animal has been affectionate before), increased aggressiveness, characteristic restlessness, loss of appetite for ordinary food, with desire to eat unusual things, intermittent disturbance of consciousness, paroxysms of fury, peculiar howling bark, rapid emaciation, paralysis, beginning in the hind limbs, death in great majority of cases in three to six days (exceptionally slightly longer) after the beginning of symptoms. (*b*) *Paralytic rabies:* short period of excitation, paralysis of the lower jaw, hoarse bark, appetite and consciousness disturbed, weakness, with paralysis spreading in great majority of cases, and death four to five days after first symptoms. There may be a number of cases showing transition types between these two forms.

In Human Beings.—*Furious Rabies.*—The first definite symptoms are difficult and gasping breath with a feeling of oppression and difficulty in swallowing, the latter, the most characteristic symptom. It is caused by convulsive contraction of the throat muscles. The attacks are brought out when attempting to drink or swallow. The very thought of drinking may bring one on; and though there is no fear of water itself, there is fear of taking it because of the effect it produces. The convulsive attacks finally become more or less general over the whole body; in certain cases some parts are more affected by reflex excitation than others; for instance, there may be slight or no photophobia, while in exceptional cases, more frequently in dogs, the hydrophobia is also absent.

Most of the special reflexes are increased. Pupils become irregularly contracted and widened until they finally remain fixed.

Human beings are seldom dangerous to the people about them: they do not make aggressive bites. In their convulsions they may bite things placed between their teeth, but not otherwise. At this time there is an increased flow of saliva, and one should avoid the contact of this with opened wounds. It may be so increased that the patient may try to get rid of it by taking it from the mouth with the hand and throwing it about. As a general thing, however, the patient has full possession of his senses between the convulsive attacks until very late in the disease.

Few changes have been noticed in the urine. The bowels are generally constipated, the temperature is increased from 38° C. to 40° C., at first with morning remissions. Just before death it may rise as high as 42.8° C. (In lower animals the temperature sinks below normal just before death.) The pulse is generally over 100 and is irregular. This stage lasts from one to four days. Death may occur during a convulsion, but more ofter there is a *paralytic stage* which lasts from two to eighteen hours. The convulsions become less frequent and the patient becomes weaker until finally there is a complete paralysis. At the beginning of this stage the patient may be able to drink water better than formerly. Death may occur at any time through paralysis of the heart or respiratory center.

Paralytic Rabies.—This form occurs quite seldom in human beings, more frequently in dogs, but not so often as a convulsive form. It is supposed to occur in humans and dogs after a more severe infection. Instead of periods of convulsions, the various muscles simply tremble and become gradually weaker until complete general paralysis supervenes. Sometimes paralysis develops very quickly and may be general before death from syncope or asphyxia occurs. This form generally lasts longer than ordinary rabies. Between these two typical forms of rabies there are many different types, giving quite different pictures of the disease.

In certain cases which have been badly bitten, treatment with protective inoculations may not save the patient, but may cause the disease to manifest itself quite late and then the symptoms may be milder than in untreated cases, though death finally results.

Length of the Disease.—The majority of the cases of furious rabies die

on the third or fourth day after the symptoms show themselves. The limits of the reported cases are one to fifteen days, though there are reports of only one or two cases dying on any day over the ninth to the fifteenth. As the time when the symptoms really begin is difficult to notice, these statistics are probably only approximately correct. In paralytic rabies the average time of death is five days.

Treatment.—The old treatment of rabies consisted simply in encouraging bleeding from the wound, or in first excising the wound and then encouraging bleeding by means of ligatures, warm bathing, cupping-glasses, etc.; the raw surface was then freely cauterized with caustic potash, nitric acid, or the actual cautery. It is doubtful whether the disease ever manifested itself after such heroic treatment if the wound were small and the treatment was begun soon after the bite; but when the wounds were numerous or extensive, the mortality was still high. As it was often impossible to apply cauterization to the wound rapidly or deeply enough to ensure complete destruction of the virus, Pasteur and others were led to study the disease experimentally in animals with the hope of finding some means of immunization or even cure; these investigations finally resulted in the discovery of methods of preventive inoculation applicable to man.

Pasteur's Method of Preventive Inoculation.—Pasteur's treatment is based upon the fact that rabic virus may be attenuated or intensified under certain conditions. He first observed that the tissues and fluids taken from rabid animals varied considerably in their virulence. Then he showed that the virus may be intensified by successive passage through certain animals (rabbits, guinea-pigs, cats) and weakened in passing through others (monkeys). If successive inoculations be made into rabbits with virus, either from the dog or the monkey, the virulence may be so exalted beyond that of the virus taken from a street dog, in which the incubation period is from twelve to fourteen days, that at the end of the fiftieth passage the incubation period may be reduced to about six or seven days. This, the strongest virus obtainable, was called by Pasteur the "*fixed virus.*" This fixed virus was used by Pasteur in his preventive treatment and has been since used as follows.

A series of spinal cords taken from rabbits dead from "fixed virus" infection are cut into short segments and suspended in sterile glass flasks plugged with cotton stoppers and containing a quantity of some hygroscopic material, such as caustic potash; these are kept at a temperature of about 22° C. The cord when taken out at the end of the first twenty-four hours is found to be almost as active as the fresh untreated cord; that removed at the end of forty-eight hours is slightly less active than that removed twenty-four hours previously; and the diminution in virulence, though gradual, progresses regularly and surely until, at the end of the eighth day the virus is inactive. An emulsion of the cord kept until the fourteenth day is made, and a certain quantity injected into the animal that has been bitten; this is followed by an injection of an emulsion of a thirteenth-day

cord; and so on until the animal has been injected with a perfectly fresh and, therefore, extremely active cord, corresponding to the fixed virus. Animals treated in this way were found by Pasteur to be absolutely protected, even against subdural inoculation with considerable quantities of the most virulent virus; and thus Pasteur's protective inoculation against rabies became an accomplished fact. As it would be undesirable to inject any but persons who had actually been bitten by a rabid, or presumably rabid, animal Pasteur continued his experiments in order to see whether it would not be possible to cure a patient already bitten. He carried on, therefore, a series of experiments which led to the discovery that if the process of inoculation be begun within five days of the bite in animals in which the incubation period was at least fourteen days, almost every animal bitten can be saved; and that even if the treatment be commenced at a longer interval after the bite a certain proportion of recoveries can be obtained. Thus the application of this method of treatment to the human subject was not tried until it had been proved in animals that such protection could be obtained and that such protection would last for at least one year and probably longer.

The chance of success in the human subject appears to be even greater than in the dog or rabbit, seeing that, on account of the resistance offered by the human tissues to the virus, the period of incubation is comparatively prolonged. Thus there is an opportunity of obtaining immunity by beginning the process of vaccination soon after the bite has been inflicted, the protection being complete before the incubation period has passed. In his earlier experiments Pasteur injected on each succeeding day emulsions from a cord dried for one day less until cords dried five days were reached; but later he used those dried for only three days. This was the "simple" ten-day method. It was soon evident that although this method was efficacious where the wounds were not severe and were confined to parts in which the nerve supply was not extensively interfered with, it was often quite inadequate in serious cases, as of wounds about the face or of wounds inflicted by a mad wolf, the virus of which is more active and the lesions made more severe than that of the rabid dog of the streets. In these latter cases the injections which, in the simple treatment, were spread over five days were made in three days; then, on the fourteenth day, a fresh series of injections or, rather, repetitions, was begun, which lasted until the twenty-first day. This was called the "intensive method."

Present Administration of Pasteur's Treatment in Human Beings.—A small portion (about 1 cm.) of the cord is rubbed up thoroughly with three cubic centemeters of bouillon until a complete emulsion is made; this is then injected by means of a hypodermic syringe, first on one side of the hypochondriac region and then on the other. With the observance of thorough asepsis no local reaction to speak of takes place nor are abscesses ever formed.

Inoculations.—The series of inoculations given in the Research

Laboratory in treating human cases after an average bite are as follows:

	Mild Treatment	Intensive Treatment for Severe Cases.
1st day,	14 and 13-day cord	12 and 11-day cord (Repeated in afternoon)
2d day,	12 and 11-day cord	10 and 9-day cord a. m., 8 and 7-day cord p. m.,
3d day,	10 and 9-day cord	6-day cord
4th day,	8 and 7-day cord	5-day cord
5th day,	6-day cord	4-day cord
6th day,	5-day cord	3-day cord
7th day,	4-day cord	2-day cord
8th day,	3-day cord	4-day cord
9th day,	2-day cord	4-day cord
10th day,	4-day cord	1-day cord
11th day,	3-day cord	4-day cord
12th day,	2-day cord	3-day cord
13th day,	4-day cord	2-day cord
14th day,	3-day cord	4-day cord
15th day,	2-day cord	1-day cord
16th day,	4-day cord	4-day cord
17th day,	3-day cord	3-day cord
18th day,	2-day cord	2-day cord
4, 3, 2, until the end (duration, 16 to 21 days).		4, 3, 2, until the end (duration, 21 to 26 days).

Some Pasteur Institutes (Berlin; Washington, D. C.) begin treatment with the eighth-day cord.

Results.—The results of Pasteur's method of protective inoculation, as recorded in the reports issued in the *Annales de l'Institut Pasteur* and those of other antirabic institutes in Italy, Russia, Roumania, etc., are very favorable. Since 1886, when the treatment was first commenced at the Pasteur Institute in Paris, over 30,000 persons bitten by rabid, or presumably rabid, animals have received preventive inoculations, with a mortality of only 0.5 of 1 per cent. The mortality of those bitten on the face or head was 1.25 per cent.; of those bitten on the hand, it was 0.75 of 1 per cent.; of those bitten on other parts of the body, a little over 0.25 of 1 per cent. As a rule, only those persons are treated who have been bitten on the face or hand or whose clothes have been lacerated so that the virus has passed into the wounds. Taking only the cases in which rabies has been confirmed in the animal by a competent examiner, the mortality of the cases treated at the Pasteur Institute in Paris is only 0.6 per cent.—a proof, it would seem, of the trustworthiness of the statistics. In view of this fact there can no longer be any doubt of the value of Pasteur's antirabic treatment. It has been stated by some that the percentage of persons killed by the bites of rabid animals is inconsiderable; but, according to the reliable statics of Leblanc, from 1878 to 1883, out of 515 persons bitten in Paris, 83 died of hydrophobia, a mortality of 16 per cent.; some authorities place the mortality at a much higher figure. According to recent statistics of Kerr and Stimson, during 1908, 111 persons died of rabies in the United States. Extensive bites on the face and head are considered to be particularly dangerous; the mortality of these is said to have been 80 per cent.

The bites of wolves seem to be more fatal than the bites of dogs or other animals; the mortality of these, in spite of the most intensive treatment, is stated to be still 10 per cent., as against a previous mortality, without specific treatment, of 40 to 60 per cent. But even Pasteur's antirabic treatment is unavailing when symptoms of the disease have manifested themselves.

On the whole, the results we have obtained in the New York Department of Health from cases treated by this method have been very encouraging.

Other Methods of Immunization.—Others methods of immunization against rabies have been proposed by different investigators. But almost all of these methods have proved on trial to be unsatisfactory and unreliable, besides being not devoid of danger. As early as 1889 Babes and Lepp conceived the idea that it might be possible by means of the blood to transmit conferred immunity against rabies from one animal to another; but although the success of these investigators was not great, Tizzoni and Schwartz, and later Tizzoni and Centanni, worked out a method of serum inoculation and protection in rabies which is worthy of attention. In this method not the rabic poison itself, but the protective material formed is injected into the tissues. These observers showed that the serum of inoculated animals is capable of destroying the pathogenic power of the rabic virus—not only when mixed with it before injection, but when injected simultaneously or within twenty-four hours after the introduction of the virus into the body.

Marie, Poor, and others have corroborated these results. The latter in our laboratory has gotten strong virus-destroying serums from hyperimmunized sheep and horses. Babes, Marie, and others now recommend treatment by sensitized virus. Poor has tried this on some of the lower animals and, though his results have been encouraging, they have not been satisfactory enough to warrant the treatment of human beings by this method.

The Cauterization of Infected Wounds.—We believe that in cases in which the Pasteur treatment cannot be applied great benefit may be derived from the correct use of cauterization with *fuming nitric acid*, even twenty-four hours after infection, and that even in cases in which the Pasteur treatment can be given, an *early* cauterization will be of great assistance as a routine practice and should be very valuable, as the Pasteur treatment is frequently delayed several days for obvious reasons, and then does not always protect. In the case of small wounds all the treatment probably indicated will be thorough cauterization with nitric acid within twelve hours from the time of infection. Our experience in dealing with those bitten by rabid animals goes to show that physicians do not appreciate the value of thorough cauterization of the infected wounds.

Pasteur Treatment by Mail.—For several years we have made a practice of sending the treatment by mail when the patients could not go for treatment. The results have been good.

Preventive Measures in Animals.—Far more important than any treatment, curative or preventive, for hydrophobia in man is the prevention of rabies in dogs, through which this disease is usually conveyed. Were all dogs under legislative control and the compulsory wearing of muzzles rigidly enforced for two years where rabies prevails, hydrophobia would practically be stamped out. This fact has been amply demonstrated by the statistics of rabies in countries (*e.g.*, England) where such laws are now in force.

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YELLOW FEVER.

Yellow fever is an acute infectious disease of tropical countries with no characteristic lesions except jaundice and hemorrhage. Other lesions that exist are those common to toxæmia.

Historical Note.—There have been many extensive studies on the etiology of this disease with numerous announcements of the discovery of its specific cause. Not one of the latter, however, has been corroborated. The *Bacillus icteroides* of Sanarelli (1897), found in the circulating blood and in the tissues of most yellow fever patients, was thought by many to be the real organism, and for some time it was the subject of most minute studies with the result that it, too, has been placed with the rejected organisms.

The epoch-making investigations of the United States Army Commission composed of Walter Reed, James Carroll, Aristides Agramonte, and Jesse W. Lazear (1901), established the truth, that this disease, like malaria, is carried from one infected person to another through the agency of a mosquito.

Finley in 1881 was the first positively to assert that the mosquito was the transmitter of the disease. He was, however, unable to prove his theory, and it remained for the commission conclusively to show that a distinct species of mosquito carried the infection.

The work of the American commission was fully corroborated by the French commission and by other workers.

The principal facts established by the commission have been summed up by Goldberger as follows:

1. Yellow fever is transmitted, under natural conditions, only by the bite of a mosquito (*Stegomyia calopus*) that at least twelve days before had fed on the blood of a person sick with this disease during the first three days of his illness.

2. Yellow fever can be produced under artificial conditions by the subcutaneous injection of blood taken from the general circulation of a person sick with this disease during the first three days of his illness.

3. Yellow fever is not conveyed by fomites.

4. *Bacillus icteroides* (Sanarelli) stands in no causative relation to yellow fever.

Though the specific parasite remains yet undiscovered, facts have been brought out by these studies which give some idea of its character.

1. It seems to require two hosts (a mammal and an arthropod) for the completion of its life cycle (analogies, *Plasmodium malariæ*, *Piroplasma bigeminum*). (The recent discovery by Stimson of a spirochete-like organism in the tubules of a yellow fever kidney is suggestive in this connection.)

2. There is a definite time between the bite of the mosquito and the infectivity of the blood (average, five days), and a definite time that the blood remains infective (three days).

3. The blood during these three days is still infective after passing through the finest-grained porcelain filters (Chamberlain B and F).

4. The blood loses its virulence quickly (forty-eight hours) when exposed to the air at temperature of 24° to 30° C. When protected from the air by oil and kept at the same temperature it remained virulent longer (five to eight days). Heated for five minutes at 55° C. it becomes non-virulent.

5. The bite of an infected mosquito does not become infectious until twelve days (at a temperature of 31° C.) after it has bitten the first patient.

The cause of the disease still remains undiscovered, notwithstanding much study of human blood and other tissues and of infected mosquitoes. The infective blood filtrates show nothing with the dark-field illumination except small motile granules similar to those found in healthy persons.

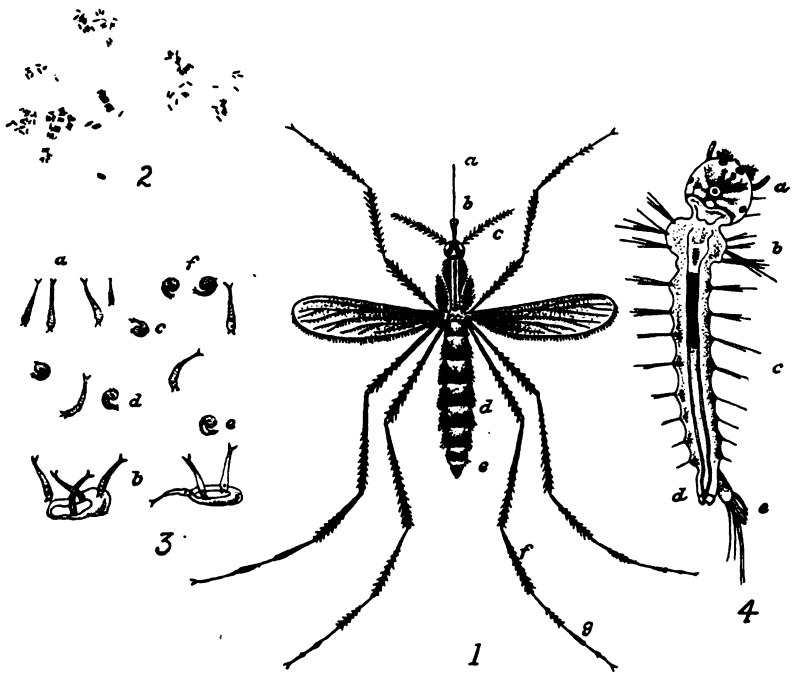
Certain facts relating to the disease seem to point to protozoa as the cause; for instance, the necessity for a second host and the long incubation time required before that host becomes infective after biting a yellow fever patient.

The higher monkeys seem to be susceptible, though no complete experiments have been made with them.

The Yellow Fever Mosquito (Fig. 196).—The name *Stegomyia* was suggested by the English entomologist Theobald, who separated this genus from the genus *Culex*, with which it was formerly classed. It was first given the specific name *fasciata*, but Blanchard proved that this had already been used and the name *calopus* (Meigen, 1818) was found to be the proper one. The salient characteristics of *Stegomyia* are: (1) The palpi in the male are as long or nearly as long, as the proboscis; in the female the palpi are uniformly less than one-half as long; (2) the legs are destitute of erect scales; (3) the thorax is marked with lines of silvery scales. *Stegomyia*, or at least *Stegomyia calopus*, is spread over a wide range of territory, embracing many varieties of climate and natural conditions. It has been found as far north as Charleston, S. C., and as far south as Rio de la Plata. There is no reason to believe that it may not be present at some time or other in any of the intermediate countries. In the United States

specimens of *Stegomyia calopus* have been captured in Georgia, Louisiana, South Carolina, and eastern Texas. The island of Cuba is overrun with this insect. The fact that *Stegomyia calopus* has been known to exist at various times in Spain and other European countries may account for the spread of yellow fever which has occurred there once or twice in former times; the same may be said of the country farther north in the United States, where *Stegomyia calopus* has not yet been reported, but which have suffered from invasions of yellow fever.

FIG. 196



Stegomyia calopus. 1. Full-grown female. × 8. 2. Eggs, natural size. 3. Larvæ and pupæ, natural size. 4. Larva. × 25 (Kolle and Wassermann).

Brackish water is unsuited for the development of *Stegomyia* larvæ. The species *Stegomyia calopus* seems to select any deposit of water which is comparatively clean. The defective drains along the eaves of tile roofs are a favorite breeding place in Havana and its suburbs; indoors they find an excellent medium in the water of cups of tin or china into which the legs of tables are usually thrust to protect the contents from the invasion of ants, a veritable pest in tropical countries. The same may be said of shallow traps, where the water is not frequently disturbed.

Like other *Culicidæ*, it prefers to lay at night. It is eminently a town insect, seldom breeding far outside of the city limits. Agramonte never found *Stegomyia calopus* resting under bushes, in open fields, or in the woods; this fact explains the well-founded opinion that yellow fever is a domiciliary infection.

The question of hibernation in the larval stage is important. Agramonte failed to get larvæ that could resist freezing temperature, and found that in the case of *Stegomyia calopus* this degree of cold was invariably fatal.

The possibility of their being capable of life outside their natural element must also be considered from an epidemiological point of view. The dry season in the countries where this species seems to abound is never so prolonged as completely to dry up the usual breeding places. Experimentally, adult larvæ removed from the water and placed overnight upon moist filter-paper could not be revived the following morning.

The question of the life period of the female insect is of the greatest importance when we come to consider the apparently long interval which at times has occurred between the stamping out of an epidemic of yellow fever and its new outbreak without introduction of new cases. The fact is that *Stegomyia calopus* is a long-lived insect; one individual was kept by Agramonte in a jar through March and April into May, in all for seventy-six days after hatching in the laboratory.

These mosquitoes bite principally in the late afternoon, though they may be incited to take blood at any hour of the day. They are abundant from March to September, and even in November Agramonte was able to capture them at will in his office and laboratory.

The mosquito is generally believed to be incapable of long flights unless very materially assisted by the wind. At any rate, the close study of the spread of infection of yellow fever shows that the tendency is for it to remain restricted within very limited areas, and that whenever it has travelled far beyond this, the means afforded (railway cars, vessels, etc.) have been other than the natural flight of the insect.

Experiments have demonstrated that not all mosquitoes which bite a yellow-fever patient become infected, but that of several which bite at the same time some may fail either to get the parasite or to allow its later development in their body. This condition is similar to that seen in *Anopheles*, with regard to malaria.

How long do infected mosquitoes remain dangerous to the non-immune community? This question cannot be definitely answered at present; there is good presumptive evidence that the mosquito may harbor the parasite through the winter and be enabled to transmit in the spring an infection acquired in the fall. There is reason to believe that the mosquito, once infected, can transmit the disease at any time during the balance of its life. Freezing temperature, however, quickly kills the insect.

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GLOSSARY.

Aggressin (L. aggressus, attacked), name given by Bail (1905) to a hypothetic substance in exudates which are produced by living organisms inoculated into animals. The substance is supposed to be an endotoxin, liberated from the bacteria through bacteriolysis. It is supposed to act by paralyzing the polynuclear leukocytes, thus preventing phagocytosis. It thereby allows the bacteria to become more aggressive, hence the name.

Alexin (*ἀλέξειν*, keep off, defend), name given by Buchner (1889) to what he believed to be the single protective substance in normal blood. The term was retained by Bordet to designate that constituent of normal and immune serums which does not withstand heating to 55° C. and which is one of the factors in lytic processes. Synonym, complement.

Amboceptor (L. ambo from *ἄμφα*, both, + capare, take), name applied by Ehrlich to that substance of the blood which withstands heating to 55° C. and which attaches itself both to the foreign cell and to the complement in order to produce lysis. It is increased during immunization. Synonyms, immune body, sensitizing substance (substance sensibilizatrice of Bordet), copula, desmon, preparator, interbody.

Amitosis (a, negative prefix, + *μιτος*, a thread, + osis), direct nuclear division without the formation of the thread-like chromosomes, asters, and spindle.

Anaphylactin (*ἀνα*, upon, back again, + *φυλάσσειν*, watch, guard), term used by Gay and Southard (1907) to designate a hypothetic substance contained in horse serum and certain other organic substances, which is an irritant to animal cells, causing them to become sensitive to the poisonous element in the organic substance.

Anaphylaxis, the term introduced by Richet (1905) for the phenomenon of sensitization to a foreign proteid, *e. g.*, guinea-pigs inoculated with horse serum become, after a period, poisoned by a second inoculation which would otherwise produce no injury.

Anisogamy (*ἀνισος*, unequal, + *γάμος*, marriage), fertilization by the union of two unequal cells.

Antagonism (*ἀνταγωνισμα*, struggling against), the opposition one organism exerts upon another either within or without the body.

Antigen (*ἀντί*, against, + *γένος*, race, stock), name given to those substances which are capable of producing antibodies. Synonym: haptin.

Autogamy (*αὐτος*, self, + *γάμος*, marriage), self-fertilization. Fertilization by the union of nuclei within the parent cell.

Bacteriolytic (*βακτήριον*, a little stick, + *λύσις*, a loosening), term describing the solvent power of blood serum for bacteria.

Blepharoplast (*βλέφαρον*, eyelid, + *πλασσειν*, mold, form), a secondary nucleus in certain protozoa, forming motor apparatus, or acting as a kinetic center.

Centrosome (*κέντρον*, centre, + *σώμα*, body), a small cell-organ which is regarded as the active centre of cell-division.

Complement (L. complementum, that which fills up completely), that constituent of normal and immune serums which is destroyed by heating to 55° C. and which unites with the immune body (amboceptor) to produce lysis.

Chitin (χιτών, a tunic), the name given by Odier to the horny organic substance which forms the integuments of insects and some other animals. Composition, $C_{15}H_{26}N_2O_{10}$.

Chlorophyl (χλωρός, yellowish-green, + φύλλον, a leaf), the yellow-green pigments common to most plants; also found in a few protozoa.

Chromatin (χρῶμα, color), the deeply staining substances of the nucleus, consisting of nuclein or nucleic acid.

Chromatophores (χρῶμα, color, + φόρος, bearing), a general term applied to the colored bodies (plastids) found in plant and animal cells.

Chromidium (χρῶμα, color + ιδιον, dim.), a name given by Hertwig (1902) to the chromatin particles which pass from the nucleus to the cytoplasm and there perform nuclear functions.

Chromosomes (χρῶμα, color + σῶμα, a body), deeply staining bodies which are formed from the chromatic nuclear network during cell division.

Commensal (L. com, together + mensa, a table), living in harmless union. One organism living on or in another without harming either.

Copula (L. copula, a bond, link), a fertilized protozoan cell.

Cytolytic (κύτος, a hollow (a cell), + λύσις, a loosening), term describing the solvent action of the blood serum on any cell.

Cytoplasm (κύτος, a hollow (a cell), + πλασμα, anything formed), that part of the cell protoplasm which is outside of the nucleus.

Ectoplasm (ἐκτός, without, + πλασμα, anything formed), the exterior denser cytoplasm of a cell.

Entoplasm (ἐντός, within, + plasm), the inner, more fluid portion of the cytoplasm.

Gamete (γιμετη, a wife; γαμετης, a husband), one of two conjugating cells, destined to die unless it unites in fertilization with another cell.

Gametocyte, the sexual cell which resolves itself into the individual gamete.

Genus (L. genus, birth, origin, race), in biology, a classificatory group ranking next above species.

Haptin (ἅπτω, to bind, + in), synonym of antigen.

Haptophore (ἅπτω, to bind, + φορος, bearing), term applied to the group of atoms which effects the specific binding to a corresponding group of atoms in certain foreign cells.

Holozoic Nutrition (ὅλος, whole, + ζωικός, animal), entirely animal-like nutrition.

Hypnocyst (ὕπνος, sleep, + κύστις, cyst), a sleeping or quiescent cyst.

Immune body (L. immunis, exempt from public service, free), synonym of amboceptor.

Isogamy (ἴσος, equal, + γάμος, marriage), the conjugation of two gametes of similar form.

Karyokinesis (κάρυον, a nut (nucleus) + κίνησις, movement, change), the series of active changes which takes place in the nucleus of a living cell in the process of division. Synonym: mitosis.

Lysis (λύσις, a loosening), the general solvent power of the blood for foreign substances.

Macrogamete (μακρός large, + γιμετη, a wife), female mating cell.

Maturation (L. maturare, to ripen), term used to designate the series of complicated processes which occur during the ripening of a germ cell.

Merzoites (μερος, a part, + ζῶον, animal, + ίτης, like), a reproductive germ produced by a protozoön without fertilization.

Metazoa (μετά, after, + ζῶον, an animal), animals ranked above the protozoa, each consisting of many cells.

Microgamete (μίκρος, small, + γαμετης, husband), male mating cell.

Mitosis (μίτος, thread, + osis), synonym of karyokinesis, so called because of the thread-like changes in the nuclear chromatin during division.

Oocyst (ὠφν, an egg, + κύστις, a cyst), fertilized cyst containing spores.

Opsonins (οχον, anything giving a zest to food; a relish), substances in blood serum which combine with the bacteria and thus prepare them for being taken up more easily by the phagocytic cells.

Parasite (παρά, beside, + σῖτος, food, live at another's table), an organism which lives on or in, and at the expense of another organism called technically the host.

Periplastic (περί, around, + πλαστος, mold, form, + ic), applied to flagella or cilia formed from the cell substance about the nucleus.

Peritrichal (περί, around, + θρίξ, a hair), applied to flagella or cilia springing from the cell membrane.

Precipitin (L. præ, before, + caput, the head, literally, falling headlong), any substance developing in the serum as the result of the inoculation of the animal with a foreign substance and which precipitates that foreign substance.

Protista (πρῶτιστα, the very first, superlative of πρῶτος, first), name proposed by Haeckel (1868) for a third kingdom, including the lowest forms of both animal and plants.

Protozoa (πρῶτος, first, + ζῶον, animal), first-formed animals; the name given to the simplest animal forms, those consisting of a single cell.

Receptors (L. re, back, + capare, take), atom groups in cells which Ehrlich conceives to have affinities for toxins and similar substances.

Reduction Division, a complicated process in maturation whereby the nuclear chromatin is reduced in amount preparatory to the formation of the gametes.

Saprophyte (σαπρός, rotten, + φυτόν, a plant), an organism that grows on decaying vegetable matter.

Schizogony (σχιζειν, cleave, split, + γονια, generation), the multiple asexual reproduction of protozoa.

Schizont (σχιζειν, cleave, split, + ont), the mother cell which gives rise to the merozoites.

Somatic (σωματικός, pertaining to the body), pertaining to vegetative growth.

Species (L. species, kind, a particular sort, etc.), a group of similar individuals which differ from other members of a genus.

Sporoblast (σπορά, seed, + βλαστός, a germ), the mother cell which gives rise to sporozoites.

Sporocyst (σπορά, seed, + κύστις, cyst), the resistant outer covering of the spore.

Sporogony (σπορά, seed, + γονια, generation), multiple sexual reproduction with the formation of spores.

Sporozoites (σπορά, seed, + ζῶον, animal, + ίτης, like), a young reproductive germ, formed in a sporoblast after fertilization.

Symbiosis (συμβιωσις, a living together), the living together of certain organisms, each of which is necessary to the other.

Syngamy (σύν, together, + γάμος, marriage), sexual reproduction.

Toxoid (τοξικόν, poison, + oid), toxin which while still combining with antitoxin has become so altered that it no longer causes poisonous effects. Ehrlich supposes the haptophore group remains intact after the destruction of the toxophore group.

Toxon (τοξικόν, poison, + on), name given to a secondary toxin produced by diphtheria or other true toxin-producing bacteria when this differs in its characteristics from the toxin of primary importance.

Toxophore (τοξικόν, poison, + φορος, bearing), term applied to the group of atoms which is the carrier of poisonous action to the cell.

Trophozoite (προφή, nourishment, ζῶον, animal, + ίτης, like), the young vegetative cell.

Virulence (L. virulentus, full of poison), the power possessed by organisms to produce injury by growth in a living host with the formation of poisonous substances. The variations in virulence of an organism in different species of hosts are due more to the ability of that organism to grow than to its ability to produce poisonous substances.

Zygote (ζυγωτός, yoked), a fertilized cell, produced by the union of gametes in lower plant or animal.

Zymophore (ζύμη, leaven, ferment, + φορος, bearing), term applied to the group of atoms which exerts a ferment action on the cell.

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