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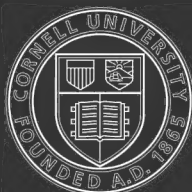
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No. 1673

BACTERIOLOGY

IN

MEDICINE AND SURGERY



A PRACTICAL MANUAL

FOR

PHYSICIANS, HEALTH OFFICERS AND STUDENTS.

BY

WM. HALLOCK PARK, M.D.,

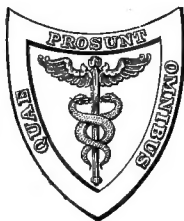
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ILLUSTRATED WITH 87 ENGRAVINGS AND 2 COLORED PLATES.



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PREFACE.

IN the following pages the attempt has been made to group together those facts in Bacteriology which will constitute a sufficient text-book for the student and which are of direct practical value to the physician and health officer. Laboratory technique is given in its essentials and to such an extent as is necessary to make bacteriological methods plain to the physician, to guide him in making the simple examinations possible in his office, and to show him under what conditions he can obtain diagnostic or other help from bacteriological examinations in laboratories. The physician can readily understand and apply the essentials of bacteriology, but the actual carrying out of the more difficult examinations should be left to the trained bacteriologist.

Such subjects as the chemical changes produced by bacteria, infection, immunity, the nature and use of protective serums, and the diagnostic value of bacteriological cultures, are particularly emphasized, since knowledge of such subjects is of special importance to

the practising physician, in that it enables him to obtain an intelligent grasp of the nature of the infectious diseases.

The methods used in the laboratory for the isolation and identification of the typhoid, tubercle, and diphtheria bacilli have been given with especial fulness, as bacteriological examinations of the discharges of persons suspected to have typhoid fever, tuberculosis, or diphtheria are now generally made for these bacteria in the laboratories of the health departments of even the smaller cities, because of the manifest importance to the public of knowing where such sources of infection exist.

In preparing this book the best works have been freely consulted. Of these, those of Flügge and Sternberg, on General Bacteriology, and those of Abbott and Mallory and Wright, on Technique, should perhaps be especially mentioned.

My sincere thanks are due to Dr. Hermann M. Biggs, the Director of the Bacteriological Laboratory, and to my colleagues in it, who have so freely furnished me with the results of their original investigations. I wish also to especially acknowledge my indebtedness to Dr. A. R. Guerard, who has given me invaluable aid in the preparation of the book. The illustrations, with the exception of those on malaria and cholera, for which I am indebted to Drs. Welch and Dunham,

are almost entirely from photographs taken from cover-glass preparations and cultures by Dr. Edward R. Leaming, Instructor in Photography in the Medical Department of Columbia University.

NEW YORK, November, 1899.

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BACTERIOLOGY IN MEDICINE AND SURGERY.

INTRODUCTION.

ALTHOUGH most of the more important discoveries in bacteriology which place it on the footing of a science are of comparatively recent date, the foundation of the study of vegetable and other micro-organisms was laid over two centuries ago. From the earliest times its history has been intimately associated with that of medicine. Indeed, it is only through the investigations into the life-history of micro-organisms in their relation to disease that our present knowledge of the etiology, course, and prevention of the infectious diseases has been acquired ; and it is only by the practical application of the principles and methods of bacteriology that many diseases can be positively diagnosed or the problems which present themselves to the sanitarian be certainly solved. The prominent position which bacteriology already holds toward medicine is, moreover, daily increasing in importance. Original discoveries are constantly adding to the list of known germ diseases, and the outlook is favorable for eventually obtaining through serums or through the toxic substances of the micro-organisms 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 preventives in many of the infectious diseases and as a cure in several. An acquaintance, therefore, with the main facts and results of bacteriology is as necessary to the education of the modern physician as a knowledge of anatomy, pathology, chemistry, or any of the allied sciences.

But before entering into a detailed consideration of the subject, it may be interesting and instructive to review briefly the most important steps which led up to the development of the science, and upon which its foundation rests, in which we shall see that the vast results obtained by bacteriology were gained only through long and laborious research, and after many obstacles were met and overcome by indomitable perseverance and accurate observation and experiment.

The first authentic observations of living microscopical organisms of which there is any record are those of Athanasius Kircher, in 1671. 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, Anthony von Leeuwenhoek observed in rain-water putrid infusions, and in his own and other saliva and diarrhoeal evacuations living, motile "*animalculæ*" of most minute dimensions, which he described and illustrated by drawings. Leeuwenhoek was a linen-draper by trade, living at the time of his discoveries in Amsterdam, but he 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. There is little doubt that Leeuwenhoek really saw some of the larger species of micro-organisms which we now recognize as bacteria, probably spirilla.

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. The first to make such an effort was Otto Friedrich Müller, in 1786 ; but having no means of obtaining pure cultures all the earlier botanists naturally fell into serious errors in the classification of bacteria. Thus various motile organisms, which are now known to be of vegetable origin, were commonly included under the infusoria, which are unicellular animal organisms.

Ehrenberg, in 1838, thus describes under the general name *Vibrioniens* four genera of filamentous bacteria :

1. *Bacterium*—filaments linear and inflexible.
2. *Vibrio*—filaments linear, sinuous, flexible.
3. *Spirillum*—filaments spiral, inflexible.
4. *Sperochæte*—filaments spiral, flexible.

Dujardin, in 1841, also placed the vibrioniens of Ehrenberg among the infusoria, describing them as extremely slender, filiform animals without appreciable organization and without visible locomotive organs.

Perty, in 1852, drew attention to the vegetable origin of these minute organisms ; Robin, in 1853, suggested their relationship to the algæ ; Davaine, in 1859, emphasized this fact ; and since it has been confirmed by the investigations of Cohn, Nägeli, and others. Bacteria are now generally believed by bacteriologists to be vegetable organisms, schizomycetes, or fission-fungi, closely allied to the algæ.

From the earliest investigations into the life-history and properties of bacteria these micro-organisms have been thought to play an important part in the causation of infectious diseases. The doctrine of *contagium aminatum* was based upon the discoveries of Athanasius Kircher and Leeuwenhoek, and the “ animalculæ ” then observed in organic materials were believed to be the cause of the great epidemics of the day, such as the plague. Shortly after these first investigations, Lange and Hauptmann advanced the opinion that puerperal fever, measles, smallpox, typhus, pleurisy, epilepsy, gout and many other diseases were due to animal contagion. Andry and Linné, in 1701, assumed the same cause for syphilis, and Lancisi, in 1718, for malaria. In fact, so wide-spread 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 pub-

lished his views in 1762, maintained that not only were all infectious diseases caused by micro-organisms, 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. He also made original investigations into the process of decomposition, and having found "*animalculæ*" in all decomposing matter, he became so thoroughly convinced of the causative relation of these organisms to the process that he formulated the law that decomposition takes place by means of living organisms, and is possible only through their increase.

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 fact that infectious diseases were of sudden occurrence, breaking out often in isolated places, and that they frequently remained clinging for long periods to certain localities, leaving others unaffected, was evidence that they were not produced by a gaseous infective agent. Moreover, 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 the probable cause being a living organism.

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 micro-organisms 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 micro-organisms 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 pre-existing organisms—the so-called vitalistic theory? This question is intimately connected with the investigations into the origin and nature of fermentation and putrefaction.

Among those who advocated the theory of spontaneous generation was Neidham, who, in 1749, attempted to prove by experiment the truth of his opinions. He placed a grain of barley in a watch-glass containing water, covered it carefully, and allowed it to germinate. On later examination he found bacteria present, which he maintained were the result of changes in the grain itself due to its germination.

In 1769, Spallanzani showed by another experiment that the theory of spontaneous generation was incorrect. He demonstrated that if putrescible infusions

of organic matter were placed in symmetrically 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.

It was objected 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. This objection was met by Spallanzani 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 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 ; whereas when no such precautions were taken with the air admitted the boiled solutions quickly fell into putrefaction, and living organisms were found to be present.

Schwann, in 1839, obtained similar results in another way ; he deprived the air admitted to his boiled liquids of micro-organisms 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.

Helmholtz, in 1843, repeated and confirmed Schwann's experiments with calcined air. He found that the free admission of air so treated to boiled organic infusions was not capable of producing fermentation of any kind.

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 micro-organisms 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, and 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 in a closed chamber, in which the air is not disturbed by currents, all suspended particles settle to the bottom, the superincumbent air being optically pure, as is proved by passing a ray of light through it. He demonstrated that the presence of living organisms in

decomposing fluids was always to be explained either by the pre-existence 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 an enormous scale in the preservation of food by the process of sterilization. Indeed, there is scarcely any biological 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 that at a temperature of 110° to 112° C. (230° to 233° F.), however, 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 that the organisms which resist the boiling temperature are, in fact, reproductive bodies, which he described under the name of "*corpuscles ovoides*" or "*corpuscles brillants*"—now known as *spores*. Perty, in 1852, and

Robin, in 1853, had observed these highly refractile bodies ; but it was not until 1876 that the development of spores was carefully investigated and explained by Cohn and later by Koch. These observers showed that certain rod-shaped organisms possess the power of passing into a resting or spore-stage under peculiar conditions of growth, and when in this stage they are much less susceptible to the injurious action of higher temperatures than when in their normal vegetative condition.

With this discovery the controversy of spontaneous generation was finally settled. If these micro-organisms, some of them being capable of producing the more resistant spores, were present in the air, dust, soil, water, etc., it was easy enough to explain the irregularities in the foregoing experiments ; nor was it any longer to be doubted that these bacteria, through their products, were the cause, not the effect, of fermentation and putrefaction, and that when organic substances were completely sterilized and protected against the entrance of living germs from without, no development of micro-organisms occurred in them.

Stimulated by the establishment of the fact that fermentation and putrefaction were due to the action of living organisms reproduced from similar pre-existing forms, the study of the causal relation of these micro-organisms to disease was taken up with renewed vigor. Reference has already been made to the opinions and hypothesis 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—*muscardine*—was due to a parasitic micro-organism. Pasteur later devoted several years' study to an exhaustive investigation into the same subject ; and in like manner Tulassee, in 1864, and Kühne, in 1855, showed that certain specific affections in grains, the potato, etc., were due to the invasion of parasites.

Very soon after this it was demonstrated that micro-organisms were the cause of certain infectious diseases in man and the higher animals. Bacteriological research has always been of special interest to physicians. Many of the most distinguished physicians of the day, in the earlier history of the science, concerned themselves in these investigations, and the progress made during the past fifteen or twenty years has been largely due to their work. Davaine, a famous French physician, has the honor of having first demonstrated the causal relation of a micro-organism 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. These experiments were subsequently confirmed by Pasteur, Koch, and others.

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 operations were made

in erysipelatos inflammations secondary to injury by Wilde, Orth, von Recklinghausen, Luthomsky, Billroth, Ehrlich, Fehleisen, and others, agreeing that in these conditions micro-organisms could always be detected in the lymph-channels of the subcutaneous tissues ; and through numerous experiments on animals the pathogenic character of the micro-organisms found in erysipelas, suppuration from wounds, diphtheria, puerperal fever, etc., was established by Oertel, Huester, Birsch-Hirschfeld, Narsiloff, Classen, Letzerich, Leber, Frisch, Eberth, Klebs and others.

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.

The next important discovery was that of Obermeier, a German physician, who, in 1873, announced having found in the blood of patients suffering from relapsing fever a minute spiral, actively motile micro-organism—the *spirochæte Obermeieri*—which is now generally recognized as the specific infectious agent in this disease.

In 1877, Weigert and Ehrlich recommended the use of the aniline dyes as staining agents in the microscopical examination of micro-organisms in cover-glass preparations.

In 1878, Koch published his important work on traumatic infectious diseases.

Hausen, in 1879, reported the discovery of bacilli in the cells of leprous tubercles, which, from subsequent researches, are believed to be the cause of leprosy.

Neisser, in the same year (1879), discovered the "gonococcus" in gonorrhœal discharges.

In 1880, Eberth and Koch independently observed the typhoid bacillus, but it was not until 1884 that Gaffky published his important researches, and proved the etiological relation of this bacillus to typhoid fever.

In the same year (1880) several important communications in bacteriological research appeared. 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.

Sternberg and Pasteur independently observed (1880) a pathogenic micrococcus in saliva, which was subsequently proved by Fraenkel and others (1885) to be the organism most commonly associated with acute croupous pneumonia—the "diplococcus pneumoniæ"—and now recognized as the usual cause of that disease.

In 1881, Koch made his fundamental researches upon pathogenic bacteria, the result of which was the establishment of a foundation upon which bacteriology of the future was to rest. 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 made, in many cases, to reproduce the specific disease of which they were the cause; and he laid down the laws by which it may be proved that a micro-organism is the specific cause of a disease. It was in the course of this work that the Abbe system of substage condensing apparatus was first used in bacteriology and that Weigert's method of staining was generally employed.

In 1882, Koch published his discovery of the tubercle bacillus.

The same year (1882) Pasteur published his investigations upon "rouget" or hog erysipelas. In this year, also, his first communication upon rabies appeared.

In 1882, also, Loeffler and Schütz discovered the bacillus of glanders.

The cholera spirillum, or "comma bacillus," was discovered by Koch in 1884.

The diphtheria bacillus was discovered during the same year (1884) by Loeffler, though it had been observed by Klebs the year before (1883).

Rosenbach, in 1884, by the application of Koch's methods, fixed definitely the characters of the various micro-organisms found in the pus from acute abscesses, etc.

The tetanus bacillus was also discovered in 1884 by Nicolaier. Carle and Rattone showed that tetanus is an infectious disease communicable to man by inoculation. Kitasato, in 1889, obtained the bacillus in pure cultures.

In 1892, Pfeiffer and Canon independently discovered a bacillus which is believed to be the specific cause of influenza.

In 1894, Kitasato, the Japanese bacteriologist, during a visit to China, discovered the bacillus of the bubonic plague.

These include all the most important pathogenic bacteria, the discovery of which is of special interest to medical students and physicians. We cannot close this brief historical review, however, of the progress of our knowledge in this department of science, without

referring to the recent discovery of the antitoxins of diphtheria and tetanus, the protective inoculations against rabies, the plague, cholera, etc., and the peculiar characteristics of the serum of those ill with infectious diseases. These discoveries, in which the names of Pasteur, Koch, Behring, Kitasato, Roux, Pfeiffer, and Widal are among the most prominent, mark an epoch in the history of bacteriology and scientific medicine. Lately, attention has also been given to the smaller group of the animal parasites, the protozoa, which may prove to be the source of infection in many diseases, such as the exanthemata, in one of which—smallpox—they have already been apparently found.

CHAPTER I.

THE GENERAL CHARACTERISTICS OF BACTERIA— THEIR MORPHOLOGY AND CHEMICAL COMPOSITION.

BACTERIA are among the smallest of all known living organisms, the largest of them having a diameter of only a few micromillimetres, while the smallest do not measure more than a fraction of a micromillimetre. Structurally and morphologically they are extremely simple, though biologically very variable. Through their ability to derive their carbon from tartrates and their nitrogen from ammonia or its salts, they are ranked in the vegetable kingdom. They obtain their food entirely through the surface absorption of soluble nutritious substances. They are reproduced by transverse division, and in some respects resemble the fungi; hence called by Nägeli fission-fungi, or schizomycetes. They are also closely allied to certain kinds of algæ, though they must receive their nourishment from living or dead organic material, since they are without chlorophyll, the green coloring matter possessed by the higher plants, by means of which they are enabled, in the presence of sunlight, to decompose CO_2 , NH_3 , and H_2S into their elementary constituents. A few varieties of unicellular organisms resemble bacteria in all their known characteristics, except that they possess chlorophyll or substances similar to it. Others, still, which have no chlorophyll, are able in the absence of light to build up organic substances synthetically. Bacteria,

especially the motile forms, are also closely allied to some of the micro-organisms which belong to the animal kingdom. If we exclude the micro-organisms containing chlorophyll, bacteria may be defined as extremely minute vegetable organisms; without chlorophyll, consisting of single spherical, rod-shaped, or corkscrew-like cells or aggregates of such cells, between whose protoplasm and nucleus it has been as yet impossible to differentiate with certainty.

Bacteria occur as saprophytes or refuse-eaters and as parasites. Saprophytic bacteria are such as commonly exist independently of a living host, obtaining their supply of nutriment from soluble food-stuffs in dead organic matter. Parasitic bacteria, on the other hand, live on or in some other organism, from which they derive their nourishment for the whole or a part of their existence. Those bacteria which depend entirely upon a living host for their existence are known as strict parasites; those which can lead a saprophytic existence, but which can also thrive within the body of a living animal, are called facultative parasites. The strict saprophytes, which represent the large majority of all bacteria, while they destroy refuse, are not only harmless to living organisms but perform many important functions in nature without which existence would be impossible, such as the destruction of dead organic material through decomposition, putrefaction, and fermentation. The parasites, on the contrary, though some of them may multiply in the secretions or on the surface of the body without injury to the animal upon which they depend for their existence, are usually harmful invaders, giving rise through the lesions brought about in the body tissues by their

growth and products to derangements which are known as acute or chronic infectious diseases.

Numerous attempts have been made by various authors to classify bacteria systematically, but usually with the proviso that the system was only a temporary one. The classification of the older naturalists and botanists was based generally upon purely morphological peculiarities. As this depended, at times, upon slight variations that were seen to occur in the size and shape of one and the same species, it naturally resulted in a more or less complicated arrangement. In this place the morphological character of the bacteria will alone be given, their classification being left until the general characteristics of bacteria have been considered.

MORPHOLOGY.

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 form of any one species may vary considerably, yet these three main divisions under similar conditions are permanent; 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 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.

The form of the bacterial cells at their stage of complete development must be distinguished from that which they possess just after or just before they have divided. As the spherical cell develops preparatory to

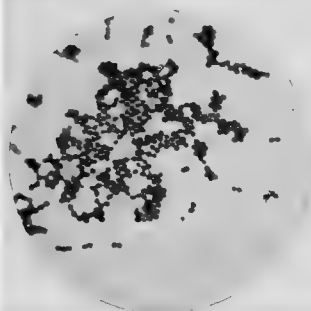
its division into two cells it becomes elongated and appears as a short oval rod; at the moment of its division, on the contrary, the transverse diameter of each of its two halves is greater than their long diameter. A short rod becomes in the same way, at the moment of its division, two cells, the long diameter of each of which may be even a trifle less than its short diameter, and thus they appear on superficial examination as spheres.

As bacteria multiply the cells produced from the parent cell have a greater or less tendency to remain attached. In some varieties this tendency is extremely slight, in others it is 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, we get different groupings of bacteria, which aid us in their differentiation and identification. Thus whether the bacterial cell divides in one, two, or three planes, we get forms built in one, two, or three dimensions. If we group bacteria according to the characteristic form of the cells, and then subdivide them according to the manner of their division in reproduction and the tenacity with which the newly developed cells cling to one another, we will have the following varieties:

1. **Spherical Form, or Coccus** (Figs. 1 to 4). 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, absolutely 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-

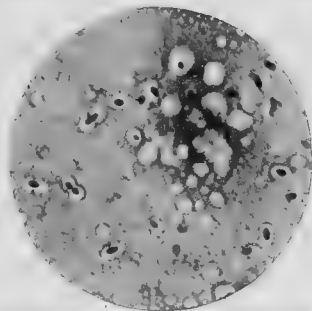
shaped forms, as frequently seen in the diplococcus of pneumonia, or the opposite, as in the diplococcus of gonorrhœa, where the cocci appear to be flattened against one another. Those cells which divide in one

FIG. 1.



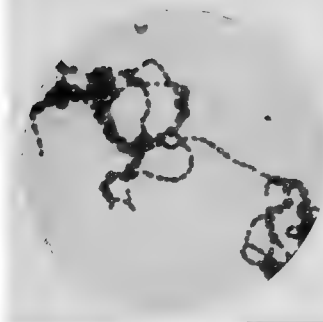
Single coccus, grouped irregularly.
Staphylococcus.

FIG. 2.



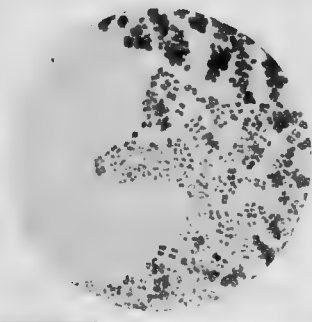
Diplococcus of pneumonia, with surrounding capsule.

FIG. 3.



Streptococcus.

FIG. 4.



Tetrads.

direction only and remain attached are found in pairs (diplococci) or in shorter or longer chains (streptococci). Those which divide in two directions, the 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 (*sarcinæ*). Those which apparently divide irregularly in any axis form irregularly shaped, grape-like bunches (*staphylococci*).

There are a considerable number of bacteria which appear to frequently assume spherical forms, or at least forms so like spheres that they cannot be differentiated from them, and yet under other conditions they generate rod-like forms. These apparently spherical bacteria we can properly regard as short forms of bacilli, which, owing to the rapidity of division, are for the time being of the same size in both diameters. Under suitable conditions, however, the true rod-shape is always developed.

2. Rod Form, or Bacillus. The type of this group is the cylinder. The length of the fully developed cell is always longer than its breadth. The size of the cells of different varieties varies 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, average over 3μ . In describing their forms bacilli are roughly classed 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 is one with a straight axis, uniform thickness throughout, and flat ends (Fig. 13, page 47); but there are many exceptions to this typical form. Thus frequently the motile bacteria have rounded ends (Fig. 10, page 43); many of the more slender forms have the long axis bent; some few species, such as the diphtheria bacilli (Fig. 5), invariably produce many cells whose thickness is very

unequal at different portions. Spore formation also causes an irregularity of the cell outline (Fig. 12).

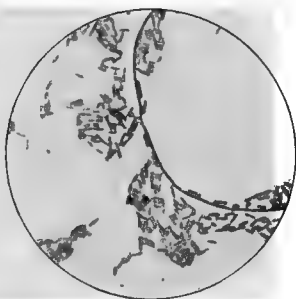
The bacilli divide only in the plane perpendicular to their long axis. A classification, therefore, of bacilli

FIG. 5.



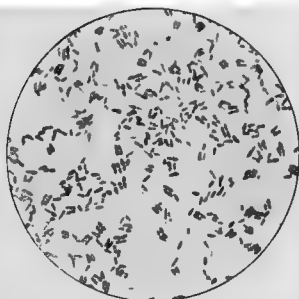
Bacilli, single and in pairs.

FIG. 6.



Bacilli, single and in threads.

FIG. 7.



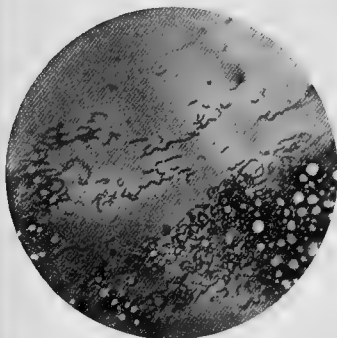
Small bacilli, mostly in pairs.

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, or as longer or shorter chains.

3. Spiral Form, or Spirillum. The members of the third morphological group are spiral in shape, or rather

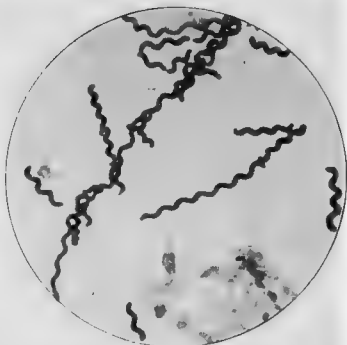
segments of a spiral. Here, too, we have large and small, slender and thick spirals. The twisting of the long axis, which here lies in two planes, is the chief characteristic of this group of bacteria. Under normal

FIG. 8.



Medium-sized spirilla.

FIG. 9.



Very large spirilla.

conditions the twisting is equal throughout the entire length of the cell. The 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.

Among uncommon morphological peculiarities in true bacteria may be mentioned *dichotomy*, or branch formation—that is, a side growth projecting from the bacterial cell. True dichotomous branching has occasionally been observed in the bacilli—viz., the bacilli of tuberculosis, diphtheria, and glanders.

Summary of Morphological Forms of Bacteria. 1. Coccus, or micrococcus. Spherical or subspherical forms.

- a.* Single coccus, grouped irregularly.
 - b.* Diplococcus, forming pairs.
 - c.* Streptococcus, forming chains, often showing paired cocci.
 - d.* Tetracoccus, forming fours by division through two planes of space.
 - e.* Sarcina, forming packets of eight members by division through three planes of space.
2. *Bacillus*. Oblong or cylindrical forms, having one dimension greater than any other, more or less straight, and never forming spirals.
- a.* Single bacillus.
 - b.* Diplobacillus and streptobacillus, forming twos or longer chains, the bacilli attached end to end.
 - c.* Filaments, or thread-like growths, in which divisions into bacilli of the normal length are not apparent, or occur irregularly and transversely, to the long axis of the growth.
3. *Spirillum*. Cylindrical and curved forms, constituting complete spirals or portions of spirals.

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

When growth is obtained upon different media, variations, especially in size, may sometimes be observed. These differences should always be described, together with a note of the media upon which they were developed and a statement as to whether such variation is a marked feature of the species under consideration.

The conditions of temperature and of nutrition which favor growth are very various for different species, so that no fixed temperature, medium, or age of growth can be determined upon as applicable to all species. Morphological 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.

It is further advisable that the appearance observed in growths developed upon a solid and in a liquid medium should be recorded.

The *structure of bacterial cells* has recently attracted considerable attention among naturalists. According to Fischer and Migula, the bacterial cells consist of a cell-membrane, a protoplasmic layer, and a central fluid; no nucleus was observed by them. In salt solutions and when dried upon a cover-glass a shrinkage of the protoplasmic layer with partial dissolution of the cell-wall occurs, due to the abstraction of water. This process is known as *plasmolysis*, and it explains the occurrence of the clear, unstained spaces so frequently seen in the stained cover-glass preparations which have erroneously been taken for spores. In water, or by the continued action of salt solution, this shrinkage does not take place. In many species of bacteria, such as the diphtheria bacilli, there is observed in the interior of the cells, on suitable staining, a peculiar granulation, to which Babés has given the name of *metachromatic bodies*, but which Ernst on more careful study has termed *sporogenous granules*.

With regard to the cell membrane, it should be noticed that it is frequently not sharply defined and often difficult to demonstrate. In many species of

bacteria, however, commonly known as *capsule bacteria*, as shown in Fig. 2, the cell membrane or the outer layers of the membrane are so much thickened that the bacteria seem to be surrounded by a gelatinous envelope or capsule, which is distinguished by a diminished power of staining with the ordinary aniline dyes. The demonstration of this capsule may be of help in differentiating between certain bacteria—*e. g.*, some forms of the streptococcus and pneumococcus. A peculiarity of

FIG. 10.



Faintly stained flagella attached to heavily stained bacilli.

the capsule bacteria is that, except very rarely, they exhibit this envelope only when grown in the animal body or in special culture media, such as liquid blood serum, bronchial mucus, etc. ; grown on nutrient gelatin, agar, or potato the capsule is only visible under very exceptional conditions, and then not distinctly.

The outer surface of bacteria when occurring in the form of spheres and short rods is almost always smooth

and devoid of appendages ; but the longer rods and spirals are usually provided with fine hair-like appendages or *flagella*, which are their organs of motility. These flagella, either singly or in numbers, are sometimes distributed over the entire body of the cell, or they may form a tuft at one end of the rod, or only one polar flagellum is found. The polar flagella appear on the bacteria shortly before division. The nature of flagella is little understood; they are believed by some to be formed of protoplasmic material which penetrates the cell membrane, and 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. 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. Unfortunately, many of the methods employed for staining flagella cause them to become degenerated, so that their perfect demonstration is often very difficult. In 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 their power be permanently lost or not we do not know.

The *vegetative reproduction* of bacteria takes place by division. When development is in progress a single

cell will be seen to elongate, in the case of spherical bacteria only slightly, and in the rod-shaped organisms considerably in one direction. Over the centre of the long axis thus formed will appear a slight indentation in the outer envelope of the cell ; this indentation increases in extent until there exists eventually two individuals. As a rule, the cells separate from one another soon after division, but occasionally they remain together for a time, forming pairs and chains. Under certain conditions of nutrition long threads or filaments are formed, which, however, when put in contact with new food, break up into fragments. At times, when the culture media are exhausted or nearly so, the bacilli and spirilla will be found to go on dividing, with little or no increase in length, and thus coccus-like forms result ; but when these are given fresh food under suitable conditions they elongate and reproduce the usual shaped organisms. According to recent investigations on the subject of cell reproduction, the division of the cell starts from the protoplasmic layer, the central space being passively destroyed, and the outer envelop is only secondarily concerned in the process. This would indicate that the central space is not a true nucleus, otherwise the division of the nucleus should precede the cell division. The complete process of cell reproduction in most varieties occupies, under favorable conditions, about twenty to thirty minutes.

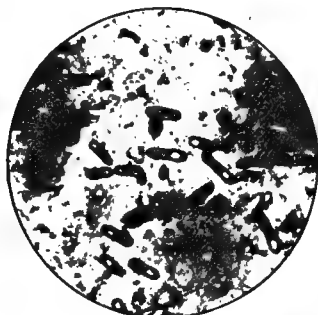
But although elongation in the greater diameter and transverse division is the rule for the majority of bacteria, there are certain groups, as the sarcinæ, for example, which divide more or less regularly in three directions. Instead of becoming separated from each

other as single cells, the tendency then is for the segmentation to be incomplete, the cells remaining together in masses. 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, division in two opposite directions results in the formation of a group of forms as tetrads. Division irregularly in all directions results in the production of clusters. 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 than is possible for them in the growing or vegetative condition. There are two kinds of spores which have been described: 1. *Endospores*, which are strongly refractile and glistening in appearance, oval or round in shape, and developed within the interior of the cell. They are characterized by the power of resisting to a considerable extent the injurious influences of heat, desiccation, and chemical disinfectants. 2. *Arthrospores*, or jointed spores, developed not within the cell but as a sprout like separation of one of its extremities. These jointed bodies are believed by some to have also greater resisting power to desiccation, etc., than the ordinary cells, though less than the endospores, and to serve the purpose of reproductive elements. Recent researches into the formation of arthrospores, however, have resulted in nothing definite, and the question of their existence even in bacteria still remains open. In describing the biological

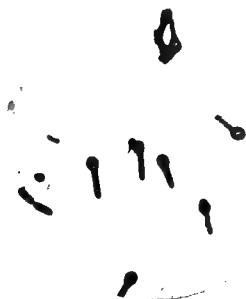
characters, therefore, of the various organisms whenever spores are mentioned, it will be understood that only the endogenous spores are meant.

FIG. 11.



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

FIG. 12.



Spores in distended ends of bacilli.

FIG. 13.



Unstained spores in centre of bacilli arranged in chains.

The production of endospores in the different species of bacteria, though not identical, is very similar. To observe the formation of spores in any species it is best to employ a streak culture on nutrient agar or a potato culture, which should be kept at the temperature nearest

the optimum of the organism to be examined. If at the end of twelve, eighteen, twenty-four, thirty, thirty-six hours, etc., specimens of the culture are observed first unstained in the hanging drops, and then, if round or oval, highly refractile bodies are seen, they should be stained for spores.

According to Fischer motile bacteria always 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 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, which are finally amalgamated into a spherical or oval refractile body. This is the spore. As soon as the process is completed there appears between two spores a delicate partition wall. For a time the spores are retained in a linear position by the cell membrane of the bacillus, but this is 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 their property of forming spores. The following are the most important spore types: (a) The spore lying in the interior of a short undistended cell; (b) the spore lying in the interior of a short undistended cell forming one of the elements of a long filament; (c) the spore lying at the extremity of an undistended cell much enlarged at that end—the so-called “head spore;” and (d) the spore lying in

the interior of a cell very much enlarged in its central portion, giving it a spindle shape.

The *germination of spores* takes place as follows : By the absorption of water they become swollen and pale in color, losing their shining, refractile appearance. Later a little protuberance is seen upon one side or at one extremity of the spore, 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. Sometimes the vegetative cell emerges from one extremity of the oval spore, and in other species the exosporium is ruptured and the bacillus emerges from the side.

In old cultures of bacteria, where the deleterious substances have developed and the food-stuffs have been largely used, there are frequently found very irregular or distorted forms, due to the abnormal development and division of the bacterial cells under the unfavorable conditions present. These are spoken of as *involution* or *degenerated* forms. If these deformed cells are placed under suitable conditions they produce again normally fashioned organisms.

CHAPTER II.

THE CHEMICAL COMPOSITION OF BACTERIA—THE CONDITIONS SUITABLE FOR THEIR GROWTH.

Chemical Composition. Qualitatively considered, the 'bodies of bacteria consist largely of water, salts, fats, and albuminous substances. There are also present, in smaller quantities, extractive substances soluble in alcohol and in ether. According to Cramer, there is no grape-sugar found in any bacterial species, but many bacteria contain amyloid substances which give a blue reaction with iodine. True cellulose has been found in the bacillus subtilis and an organism closely allied to the bacillus coli; the tubercle bacillus also forms cellulose in the animal body, though no cellulose has been found in cultures of the tubercle bacillus. But from these and from cultures of a "capsule bacillus from water," allied to the pneumococcus of Friedländer, large quantities of a gelatinous carbohydrate similar to hemi-cellulose have been obtained. Nuclein, first demonstrated by Vanderville, is only found with difficulty; but the nuclein bases—xanthin, guanin, and adenin—have been found in considerable amounts. There is a group of bacteria which contain 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 com-

position 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 ash, depend largely on the composition of the media. Thus the 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. Beside the concentration of the culture, its temperature and age also influence the amount of residue and ash produced. The residue varies, moreover, in its composition in the same species under the influence of the culture media employed. Thus the Friedländer pneumonia bacillus grown on nutrient agar containing peptone yields of residue :

	With 1 per cent. peptone.	With 5 per cent. peptone.
Nitrogenous matter	71.7 per ct.	79.8 per ct.
Extractives	10.3 "	11.3 "
Ash	13.9 "	10.3 "

	With 1 per ct. peptone + 5 per ct. glucose.
Nitrogenous matter	63.6 per ct.
Extractives	22.7 "
Ash	7.8 "

It would thus appear 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.

The cholera spirillum shows still greater variations in the residue when grown in soda bouillon containing albumin than in Ushinsky's medium, which is free from albumin. Thus Cramer found as an average

yield of residue from five different varieties of cholera spirilla: Albumin 65 per cent. and ash 31 per cent. when grown in soda bouillon, while in Uschinsky's solution there was only 45 per cent. albumin and 11 per cent. ash. The five varieties of spirilla which in soda bouillon yielded almost exactly the same quantities of albumin and ash, in the other medium free from albumin exhibited a very variable composition. This shows how little dependence can be placed upon any single chemical or cultural reaction for the differentiation of two species of bacteria. Judging from the percentage composition of the cholera spirilla when grown in the Uschinsky medium, these five varieties, taken from Paris, Hamburg, Shanghai, etc., might be considered to be different species, whereas they were probably merely varieties of the same species of bacteria.

CONDITIONS OF GROWTH.

Culture Media. Although there are among the bacteria related to disease a number which are met with only in the bodies of living animals or plants, and, therefore, so far as we know, strictly parasites, yet most pathogenic bacteria can be cultivated more or less readily in artificial culture media under suitable conditions, as, for example, the tubercle bacillus and the gonococcus. The majority of bacteria which occur usually as saprophytes are easily cultivated artificially; but there are some, such as various micro-organisms found in the saliva and in water, which, with our present knowledge, are either difficult or impossible to cultivate.

All bacterial culture media must contain an abun-

dance of water ; salts are also indispensable, and there must be organic material as a source of carbon and nitrogen. The greater number of important bacteria and all the pathogenic species thrive best in media containing albuminoid substances and of a slightly alkaline reaction. The demands of bacteria in the composition of the culture media vary very considerably. There are some species of water bacteria, for instance, which require so little organic material that they will grow in water that has been twice distilled. In such cases development probably takes place owing to some contamination of the water, or else through the decomposition of the ammonia and carbonic acid in the air. A certain species will grow abundantly in water containing ammonium carbonate in solution and no other source of carbon and nitrogen. This shows the power of some bacteria of producing cell substance from the simplest materials—a power which belongs to the higher plants which obtain their nourishment from the air through their chlorophyll and the assistance of sunlight. Few bacteria, however, of any importance in medicine are 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

abuminoid material and less easily from ammonium compounds. Their carbon they derive from albumin, peptone, sugar and 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; thus some derive it from carbolic acid in very dilute solutions. Another species of bacteria isolated by Winogradsky were shown by him to derive their carbon from CO_2 .

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, material 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., for the development of bacteria will be dealt with in a later chapter.

The relation of bacteria to oxygen: The majority absolutely require oxygen for their growth, but a considerable minority fail to grow unless it is excluded. A knowledge of this latter fact we owe to Pasteur, who divided bacteria into aërobic and anaërobic. Between these two groups we have those that can grow either with or without the access of oxygen.

Some at least of the strict anaërobic bacteria require

for the full development of their life functions the presence of fermentable substances, such as sugars, from which they obtain oxygen. Among bacteria can be found all gradations between those bacteria which develop only in the presence of oxygen to those which develop only in its absence. In so far as for any variety the amount of oxygen present is unfavorable there will be more or less restriction in some of the life processes of these bacteria, such as pigment and toxin production, spore formation, etc. It has also been found that some, at least, of the aërobic bacteria can be accustomed to grow without oxygen, and that some of the anaërobics can be accustomed to grow with it.

Sulphur and phosphorus are two important food-stuffs required by bacteria. Either calcium or magnesium and sodium or potassium are also usually required for bacterial growth. Iron is demanded by but few varieties.

When we consider the more complex culture media, either those naturally existing, such as blood-serum, or those created by us for the cultivation of bacteria, we find, beyond the necessary amount of soluble food-stuffs, that the relative proportion of each form and the total concentration are of great importance. It is, nevertheless, true that very wide differences can exist with but slight effect upon the development of bacteria, the development of the bacteria usually ceasing through the accumulation of deleterious substances in the culture media rather than through food exhaustion.

The reaction of the nutritive media is of very great importance. Most bacteria grow best in those that are slightly alkaline or neutral. 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.

The influence of one species upon the growth of another, either when the bacteria grow together or follow one another, is very marked. The development of one variety of bacteria in a medium causes that substance, in the majority of instances, to become less suitable for the growth of other bacteria. This is due partly to the impoverishment of the food-stuff, but more to the production of chemical substances or enzymes, which are antagonistic not only to the growth of the bacteria producing them, but to many other varieties also; less frequently the changes produced by one variety of bacteria in the food-stuff are favorable for some other form.

For the growth of bacteria a suitable temperature is absolutely requisite. For different varieties the most favorable temperature varies, but for all a range of about $2\frac{1}{2}^{\circ}$ C. above or below this most favorable point covers the limits for their most vigorous growth. Few bacteria grow well under 10° C. and few over 40° C.; 2° C. is about the lowest temperature that any bacteria have been found to grow and 70° C. the highest.

In many instances the temperature of the soil in which the bacteria are deposited is the controlling 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; while exposure to higher temperatures than allows of growth destroys the life of the bacteria. The relations of the temperature to bacterial life and death will be dealt with fully in a later chapter.

CHAPTER III.

VITAL PHENOMENA OF BACTERIA—MOTION, HEAT AND LIGHT PRODUCTION—CHEMICAL EFFECTS.

Motility. Many bacteria when examined under the microscope are seen to exhibit active movements in fluids. This motility is produced by the fine hair-like flagella attached to all motile species. The movements are of a varying character, being described as creeping, waddling, rotary, undulatory, sinuous, snake-like, 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—a dancing, trembling motion possessed by all finely divided organic particles. If in doubt in such cases it is best, where the matter is of importance and one is skilled in the technique of staining, to stain the organisms for flagella, and also to examine them in a 0.1 per cent. bichloride of mercury solution, when, if the movements continue, they are purely molecular. Not all species of bacteria which have flagella, however, exhibit at all times spontaneous movements; such movements may be absent in certain culture media and at too low or too high temperatures, or of either an insufficient or excessive supply of oxygen.

Some chemical substances seem to 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 chemotaxic 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. Substances showing positive chemotaxis for nearly all bacteria are peptone, urea, and very weak solutions of bichloride of mercury. While among those showing negative chemotaxis are alcohol and many of the metallic salts.

The 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 seawater, 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. Thus, cold paralyzes them and interrupts their power of emitting light. High temperature, acids, chloroform, etc.,

inhibit for a time or destroy this property. Living bacteria are always found in phosphorescent cultures; a filtered culture free from germs is invariably non-phosphorescent; but while the organism cannot emit light except during life, it can live without emitting light, as in an atmosphere of carbonic acid gas, for instance. Most organisms require, in order to be able to emit light, the presence of peptone and oxygen, and many also need carbon and nitrogen. 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 one partly at least due to the action of bacteria. Rabinowitsch suggests that very probably the high temperature which is here exhibited is caused in part by the so-called thermophilic bacteria, but there are no accurate observations as to the true source of this heat.

Chemical Effects. The processes which bodies being split up undergo 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 and also to produce fermentation products or poisons, and they are able to do these things either analytically or synthetically with almost equal ease. This ambidextrous metabolic 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. Life processes carried on without oxygen do not effect any profound molecular changes in the organic material which is broken up; but in order that the living organism may obtain the requisite quantities of energy from this mode of life, a proportionately large amount of material must be superficially disintegrated. Therein lies the mechanical foundation for the power of a small amount of ferment to cause the production of much alcohol or lactic acid, and that parasites which have invaded the living body can generate intensely poisonous substances out of the body proteids.

In the presence of oxygen 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. Some bacteria have adapted themselves to the exclusive use of compound oxygen, using those compounds from which oxygen can be obtained, and others—the obligatory anaërobes—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. Since, under strictly anaërobic conditions, any secondary oxidation of the primary decomposition products is impossible, the latter accumulate without formation of by-products. Many parasitic bacteria are found to produce far more poison in the absence of air than in its presence.

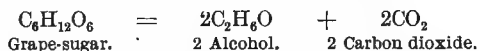
Organized and Unorganized Ferments. All the chemical effects of bacteria are largely dependent upon the composition of the culture media. Thus many species of bacteria which in albuminous media produce no

visible change, when sugar is added decompose it, with the production of gas. The term fermentation is differently used by different authors. Some call every kind of decomposition due to bacteria 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 living organisms or substances contained within them (organized ferments), or by chemical substances thrown off from the bacteria (unorganized or chemical ferments or enzymes). In the first the action is due to the growth of the organisms producing the ferment,¹ as in the formation of acetic acid from alcohol by the action of the vinegar-plant, and in the second the enzyme causes a structural change without losing its identity, as in digestion. These enzymes 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. We can only speak of chemical ferments when it can be demonstrated that the fermentation continues in the absence of all living bacteria.

¹ Buchner (*Berichte d. deutsch. chem. Gesellsch.*, xxx. 117-124 and 1110-1113) has shown that even in those cases of fermentation in which, until lately, we have believed the organized cell itself was necessarily concerned that the cell protoplasm squeezed from its capsule 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 in the cell. The increase of both ceases with the death of the bacteria producing them.

This may be accomplished by the addition of disinfectants—carbolic acid, chloroform, ether, etc.—to the cultures or by filtration. Ferments, like albuminoids, are non-dialyzable. They withstand dry heat, but are destroyed in watery solutions by a temperature of over 70° C. They are injured by acids, especially the *inorganic* ones, but are resistant to all alkalies. All fermentation has for its object the acquisition by the organism of a store of energy. This is accomplished in either of the ways above mentioned. The simplest and commonest example of decomposing fermentation produced by an enzyme is that of sugar :



or,



or,



Bacteria which develop in the absence of oxygen are especially in need of this source of oxygen. Anaërobic bacteria, for this reason, have the power of decomposing sugar, while many facultative anaërobes are only capable of producing fermentation when oxygen is excluded.

Opposite to this, and far less common, is oxidizing fermentation, as in the production of acetic acid from alcohol. Here the energy is acquired not by the decomposition but by the oxidation of the alcohol.

The proteolytic or peptonizing ferments which are somewhat analogous to pepsin and 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. It is not pepsin, as pepsin acts only in the presence of acid, and gelatin is liquefied with an alkaline reaction only. The production of proteolytic ferments by different cultures of the same varieties 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 varieties have been repeatedly found. These observations have detracted considerably from the value in cultures of the property of liquefying gelatin as a positive diagnostic characteristic. Most conditions which are unfavorable to the growth of bacteria seem to interfere also with their liquefying power.

Certain bitter-tasting products of decomposition are formed by liquefying bacteria in media containing albumin, as, for example, in milk.

Diastatic ferments convert starch into sugar. That these are produced by bacteria is shown by mixing starch paste containing 1 per cent. thymol with cultures to which 1 to 2 per cent. thymol has been added, and keeping the mixture 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. Bacteria may be directly tested for sugar also by boiling potato-broth cultures and using the extract.

Inverting ferments—that is, those which convert cane-sugar into grape-sugar—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 albumin.

Rennet 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. These ferments have not been thoroughly investigated; they are probably present, however, in all species of bacteria which coagulate milk with the production of acid.

Fermentation yields products that are poisonous to the ferment; hence fermentation ceases when the nutriment is exhausted or the fermentation is in excess. Different kinds of fermentation obtain specific names, according to product. Thus *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.

Pigment Production. Pigments have been little chemically studied, but the recent investigations of Klein and Migula, Thumm and Schneider, and others throw some light on the subject. They have no known importance in connection with disease, but are of interest and have value in identifying bacteria.

RED AND YELLOW PIGMENTS. Of the twenty-seven red and yellow bacteria studied by Schneider, almost all produce pigments soluble in alcohol and insoluble in water. The larger majority of these 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 egg, 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 are also produced by the so-called fluorescent bacteria, along with a pigment named bacterio-fluorescein. In cultures the fluorescence is at first blue; later, as the cultures become alkaline, it is green.

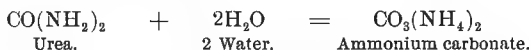
Numerous investigations have been made to determine the cause of the variation in the chromogenic function of bacteria. All conditions which are unfavorable to the growth of the bacteria decrease the production of pigment, as cultivation in unsuitable media or at too low or too high a temperature, etc. The *B. prodigiosus* produce no pigment at 37° C., and when transplanted at this temperature, even into favorable media, the power of pigment production is gradually lost.

Otherwise colorless species of bacteria sometimes produce pigments. Thus yellow to red colonies of the pneumococcus have been observed, and colored varieties of the streptococcus pyogenes. Occasionally colored and uncolored colonies of the same species of bacteria may be seen to occur side by side in one plate culture, as, for example, the staphylococcus pyogenes.

Alkaline Products and the Fermentation of Urea. Aëro-bic bacteria sometimes produce alkaline products from albuminous substances in culture media free from sugar.

Most species of bacteria produce acids in the presence of sugar, which explains the fact that neutral or slightly alkaline cultures become acid at first from the small amount of 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 after the acid formation has ceased. The substances producing the alkalinity in cultures are chiefly ammonia, amine, and the ammonium bases.

The conversion of urea into carbonate of ammonia affords special evidence of the production of alkaline substances by bacteria:



Leube has isolated several organisms from putrefying urine which separate ammonia from urea. The power of decomposing urea, however, is not wide-spread among bacteria. Out of twenty-seven organisms studied by Warington, only two were found to decompose urea. Of sixty species investigated by Lehmann, three only developed the odor of ammonia from sterilized human urine.

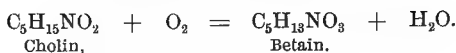
Ptomains—Toxins. But beside ammonium carbonate, a large number of basic crystalline substances have been recognized, especially by Brieger, as products of bacterial growth. These are now commonly known as *ptomains*, or putrefactive alkaloids (from $\pi\tau\tilde{\omega}\mu\kappa$, putrefaction).

Nencki, and then later Brieger, Vaughan and others, succeeded in preparing organic bases of a definite chemical composition out of decomposing fluids—meat, fish, old cheese, and milk undergoing bacterial decomposition—as well as from pure bacterial cultures. Some of these were found to exert a poisonous effect, and for a long time

were looked upon as the specific bacterial poisons, while others were harmless. The poisons are particularly interesting, since they may be present in the decomposing cadaver (hence the name ptomaïn), 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 are now known not to be the substances to which are due the specific poisonous effects of bacteria which are designated as toxins, and have entirely different characteristics.

Many ptomains are known already and the empirical formula of each made out, and among them are some whose exact chemical composition is established. The first of these bodies to be separated was colloidin ($C_8H_{11}N$), obtained by Nencki from putrefying gelatin. Another, trimethylamin ($C_3H_9N = (CH_3)_3N$), gives an odor like herring-brine. 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 fatty substance found in brain tissue and other nervous tissue.

By the oxidation of cholin there can be produced the non-poisonous betain or trimethylglycocoll occurring in beet-juice, and the highly toxic muscarin, found by Schmiedeberg in a poisonous toadstool and by Brieger in certain decomposing substances :



The ptomain tyrotoxin, obtained from cheese by Vaughan, is apparently derived from butyric acid.

Pyocyanin ($\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}$), which produces the color of blue or blue-green pus, and has been regarded by Læderhose as related to the coal-tar products, 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, where they play the rôle of self-poisons, or leucomains. Some believe the symptoms designated as coma and tetany may be ascribed to the absorption of substances of this nature. Since the name ptomain was given to the poisonous products of bacterial growth before these products were chemically understood, and even now, when the name is restricted to crystalline bodies, it is by many frequently applied to all bacterial poisons, as in cases of poisoning due to decomposing meat or sausage or to cheese or milk. Instead of ptomains these may be due to the poisonous proteids or toxins. Such poisonous proteid bodies are always formed in the beginning of decomposition processes. Some of the ptomains obtained by chemists are due not to putrefactive changes but to the chemical methods used to obtain them.

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 con-

sistency. This is then dissolved in 96 per cent. alcohol, freed from albumin and other contamination by an alcoholic solution of lead acetate, the lead precipitated, the filtrate concentrated, and again precipitated by an alcoholic solution of mercuric chloride, which forms a double mercury compound with the ptomain. The alcohol is evaporated by heat, the mercury separated by sulphuretted hydrogen, and a double compound formed with gold and platinum, the crystallizability of which permits of its purification; or the crystalline hydrochloride is directly obtained, and the free bases, which are often liquid, separated by means of sodium hydrate.

Many of these ptomains, like most vegetable alkaloids when they are set free by sodium or potassium hydrate, are obtainable by agitation with ether in aqueous solution; but Brieger's method is preferable, because many substances not taken up by ether are here extracted.

Complex Albuminoid Poisons—Toxalbumins or Toxins. These may be divided into two classes:

1. **BACTERIAL PROTEINS** (Buchner). By these are understood poisonous substances of a proteid nature produced by bacteria which are not affected by heat, which are capable of producing fever (pyogenic) and causing inflammation (phlogogenic), and which can be obtained by boiling for several hours potato cultures treated with an 0.5 per cent. solution of potassium hydrate (about 50 volumes of potassium hydrate to 1 volume of bacterial substance). From the clear, filtered liquid the proteins are precipitated by weak acid, carefully added, and the precipitate washed and dried; before use they can be dissolved in weak soda solution.

The best known protein is Koch's old *tuberculin*;

mallein is another. According to Buchner and Römer, all bacterial proteins are very similar in their action, and Mathes maintains that deuterio-albumose, which is obtained by the action of pepsin on albumin and has no connection with bacteria, has an effect on tubercular guinea-pigs somewhat similar to tuberculin.

Toxins—Active Proteids. TOXALBUMINS. Fraenkel and Brïeger, confirming the statements of previous investigators (Christmas, Roux and Yersin, and Hankin), have shown that amorphous poisons having an intense and often specific toxic action—that is an effect similar to that produced by infection with the living organism—may be precipitated from bouillon cultures by agents precipitating albumin. They therefore called these poisons “toxalbumins,” and regarded them as being analogous to the toxalbumins of vegetable origin, like ricin from the castor-oil bean (*Ricinis communis*), and abrin from the jequirity bean (*Abras precatorius*). The majority of investigators, however, consider these poisons to be “labile”¹ albuminous substances, which are derived from the bacterial cells. Some have assumed that they were similar to snake poisons or to the enzymes. Like these substances, they are very sensitive to the action of heat, chemical agents, light, etc.

Toxalbumins are obtained as crude substances by the precipitation of the products of fully developed cultures in bouillon under a vacuum, with absolute alcohol or ammonium sulphate, after the culture fluid has been freed from living germs by its passage through a porcelain filter. When ammonium sulphate is used the precipitate is freed from this reagent by dialysis

¹ So-called “labile” substances are such as are prone to undergo chemical changes or alterations of atomic structure; unstable.

through parchment against running water, and after concentration the substances are again precipitated by absolute alcohol. Recently it has been found that zinc chloride separates these bodies quantitatively, and that the toxins may be obtained from this precipitate by means of sodium phosphate (Brieger and Boer).

All along, however, some doubt has been expressed as to whether these so-called toxalbumins were really only obtainable by precipitation from albumin or whether they had anything to do with albumin at all. With regard to tetanus poison, Brieger and Cohn have now succeeded in obtaining what they consider an almost pure toxin from the crude poison by means of acetate of lead and ammonia. This substance gives a slight violet color with copper sulphate and soda solution, but otherwise no albumin reaction; it contains neither phosphorus nor sulphur, and is apparently not an albuminous substance. The statement previously made by Uschinsky that he had obtained albuminoid tetanus and diphtheria poisons in culture media devoid of albumin could not, heretofore, be confirmed, owing to the difficulty experienced by most investigators in getting a sufficient growth of these organisms on such media. Brieger and Cohn have found that cholera spirilla produce a non-albuminous poison in Uschinsky's culture media (free from albumin); and now diphtheria toxin has been recognized to be non-albuminous (Brieger and Boer). It is becoming more and more customary to call proteid bacterial poisons simply *toxins*, irrespective of their composition, and to ignore the existence of the above-described crystallizable toxins of simple constitution.

With regard to the other properties of these toxins,

taking tetanus toxin as an example, it may be said that in aqueous solution it is not coagulated by heat, but is in time deprived of its poisonous qualities. The addition of small quantities of acids or alkalies to the solution, and the continued passage through it of carbon dioxide or sulphuretted hydrogen, distinctly reduce its toxicity. When exposed to light and air, either in a dry state or in solution, the toxin deteriorates rather rapidly. It withstands a temperature of 70° C. for some time without being wholly destroyed; higher temperatures decompose it rapidly. When protected from the light and air it is slowly converted into an inactive substance; it is better preserved under absolute alcohol, pure ether, and the like. The toxicity of the purest tetanus toxin now obtainable is almost incredible: 0.00005 milligramme of it kills a mouse of 15 grammes; a man of 150 pounds weight, if he were equally susceptible, would be killed with 0.23 milligrammes. It requires 30 to 100 milligrammes of strychnine to kill a man under ordinary circumstances. The most virulent diphtheria bacilli produce a specific poison which does not fall far behind that of tetanus in power.

Sulphuretted Hydrogen. Sulphuretted hydrogen is a very common bacterial product. Its presence is determined by pasting 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.

Sulphuretted hydrogen may be formed:

1. From albuminous substances. This power, according to Petri and Maassen, of forming sulphuretted hydrogen, particularly in liquid culture media containing much peptone (5 to 10 per cent.) and no sugar, is possessed, though in different degree, by all bacteria examined by them; 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.

2. From powdered sulphur. All bacteria produce in culture media to which pure powdered sulphur is added considerably more H_2S than without this addition. Petri and Maassen suggest that this is due to the nascent hydrogen produced by the bacteria.

3. From thiosulphates and sulphites. Studied particularly in yeast, but demonstrated also by Petri and Maassen in several bacteria.

The presence of sugar in the culture does not affect the production of H_2S by bacteria, but saltpetre reduces it, nitrites being formed. The absence of oxygen favors the production of H_2S . Light diminishes the development of H_2S by facultative anaërobes, sulphates being formed instead.

Reduction Processes. All bacteria, as we have seen, possess the property of converting sulphur into sulphuretted hydrogen, for which purpose is required the presence of nascent hydrogen. The following processes depend also in part upon the action of nascent hydrogen:

1. 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, acid being formed, the litmus pigment is turned red. According to Cohn, the property of reducing litmus belongs to all liquefying bacteria, but some non-liquefying species also possess it.

2. The reduction of nitrates to nitrites and ammonia. The first of these properties seems to pertain to a great many bacteria—at least Petri and Maassen found in six species, grown in bouillon containing 2.5 to 5 per cent. peptone and 0.5 per cent. nitrate, that almost all produced nitrite abundantly; once only was ammonia observed. In a number of bacteria studied by Rubner only one failed to produce nitrite. 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,

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 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.

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 beside cholera. 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 a 0.5 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. Levandoosky states that the color group in general, glanders, diphtheria, proteus vulgaris, and most of the spirilla, are indol producers; with the exception of the spirilla, these bacteria also produce phenol.

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 fermentation by the lactic acid of 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 complex organic compounds, albuminous substances, and the like (glue, albuminoid bodies), 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, and, finally, sulphuretted hydrogen, mercaptan, carbonic acid, hydrogen, and, possibly, marsh-gas (H_4C).

According to recent observations, nitrification is produced by a small, special group of bacteria, cultivated with difficulty, which do not grow on our usual culture media. From the investigations of Winogradsky it would appear that there are two common micro-organisms 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 special nitrate-fermenting bacteria, however, were first accurately described by Barri and Stutzer. In their exhaustive investigation they first isolated from horse-manure two bacteria, neither of which was alone capable of producing nitrogen from nitrates, but which together in the presence of oxygen, but never without it entirely, decomposed nitrates energetically. Later a second denitrifying bacillus was found, *B. denitrificans* II., which by itself was able to produce nitrogen from nitrates.

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.

Nitrogen Combination. The *bacillus radicum* of Beyerinck, which was isolated by him, has the power of assimilating nitrogen from the air. This bacillus is found in the small root-nodules of various leguminous plants (pease, clover, etc.), and can be obtained from these in cultures. Different varieties exist in different kinds of legumes, each kind of legume apparently having a special variety of bacteria adapted to it, and not every variety is capable of producing nodules in all legumes. There are certain "neutral" varieties, however, existing free in the soil and not adapted to any special legume, and these seem to be able to form nodules in different legumes.

By the aid of these root-bacteria, which gain entrance to the roots and there produce this nodular formation,

the leguminous plants are enabled to assimilate nitrogen from the atmosphere, thus yielding harvests of grain, etc., which are highly nitrogenous, upon soils which are naturally poor in nitrogen. This explains the reason why poor, sandy soils become gradually fruitful when pease, lupine and other varieties of legumes are grown upon them and then turned under with the plough. 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 (Stutzer).

Formation of Acids from Carbohydrates. Free acids are formed by many bacteria in culture media containing sugar; the production of acid in ordinary bouillon takes place on account of the presence of grape-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 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, a similar change in reaction now takes place to that in ordinary culture media in the absence of sugar—viz., the acid is neutralized gradually, and in the end the reaction becomes 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.

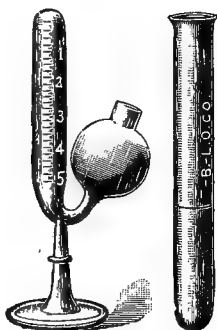
Some bacteria also seem to have the power of decomposing cellulose, found in the stomach and intestinal contents of herbivorous animals and in marshy soils, with the production of marsh-gas.

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, according to Smith, is always mixed with H_2 . 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 glucose-agar, containing about 1 per cent. grape sugar, 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.

For the determination of the quantity and kind of gas produced by a given micro-organism the fermentation tube recommended by Theobald Smith is the best. This is a bent tube, constricted greatly at its lowest portion (Eichorn's), supported upon a glass base, as shown in Fig. 14. The graduation shown in the

FIG. 14.



Fermentation tube left side, ordinary tube on right side.

upright arm is not essential for ordinary laboratory work. The tube is filled with a culture media consisting of 1 per cent. glucose, peptone bouillon (without air-bubbles), 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 *aërobie* species; if this

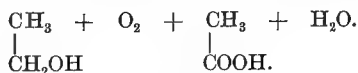
clouding occurs only in the closed arm, while the open bulb remains clear, it is an anaërobic species.

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 thoroughly 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 new volume of gas formed is permitted to escape. That which passes off is carbonic acid gas; the remainder is nitrogen, hydrogen, and marsh-gas.

For the quantitative determination of these gases Hempel's gas pipettes may be used. The principle of the method is this: The hydrogen, mixed with oxygen and passed over red-hot palladium asbestos, becomes water, and thus disappears; the carburetted hydrogen is converted into carbon-dioxide and is estimated as such; the remainder is nitrogen.

Formation of Acids from Alcohol and Other Organic Acids. It has long been known that the bacterium *aceti* and other allied bacteria convert dilute solutions of ethyl-alcohol, under the influence of oxidation, into acetic acid:



The higher alcohols—glycerin, dulcitol, mannitol, etc.—are also converted into acids—glyceric, indeed, as commonly as sugar.

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 in various acids by the action of bacteria, such as butyric, priprionic, valerianic, and acetic acids; also succinic acid, ethyl-alcohol, and, more rarely, formic acid, have been produced. Among the gases formed were chiefly CO_2 and H_2 .

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

CHAPTER IV.

THE RELATION OF BACTERIA TO DISEASE.

IN the preceding chapter our consideration has been given largely to the chemical effects of bacteria on dead organic substances. Here we have to consider the growth of bacteria in living bodies 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 considered as merely a quantity of organic and inorganic material, to be used for food for bacterial growth, still the fact that bacteria do increase in the living body shows that its tissues are under certain conditions a suitable nutrient soil for their growth. In a sense, therefore, we are warranted to consider the living body as we do any other medium for bacterial growth, remembering, however, that beside the chemical nature, temperature, etc., of its tissues, micro-organisms have also to reckon with the mysterious influence of life with which all parts of the body are endowed. In the production of disease by micro-organisms there are two main factors involved—viz., the power to elaborate poison and the ability to multiply. 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 there any variety which if it multiplied in the body to the full extent to which it is capable under favorable con-

ditions will not produce disease. As already mentioned, bacteria even under similar conditions differ enormously in the amount of poison which each organism produces and in their ability after gaining entrance to multiply in the body.

To understand at all the production of disease through bacteria we must recognize that both the body invaded and the bacteria which invade are living organisms. They are in bulk, wide apart, but both have life. Just as there are different races and species of animals, 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. Considering these facts, we can readily understand how all of bacteria do not grow equally well in every variety of animal, nor even find the body of the same animal always equally suitable. This is all the more apparent when we consider that the study of bacteria in the more simple and known conditions of artificial culture media has already shown us how extremely sensitive many bacteria are to slight chemical, thermal, and other changes.

Thus if we take specimens of diphtheria bacilli from three different cases of diphtheria, we find that on growing them for several days in suitable bouillon one will have produced poison in the culture fluid to such a degree that one 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. In other words, different varieties of diphtheria bacilli under similar conditions have different toxin-producing powers.

Let us now cultivate these same bacilli 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 the 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 shows that growth of bacteria may occur in the body and yet no specific poison be produced, and that of the same species of bacteria some varieties are capable of producing toxin under less favorable circumstances than others.

Slight variations in the culture media are, moreover, of great importance in aiding or inhibiting the growth of bacteria. Thus the diphtheria bacillus grown in neutral bouillon containing a little glucose will at first thrive luxuriantly; but as the result of its growth fermentation of the glucose takes place and acid is produced, which then inhibits the further development of the bacillus. After a while, however, the glucose having been entirely destroyed, acid formation ceases while alkaline products continue to be formed, and thus render the medium neutral or slightly alkaline again, and now a vigorous growth again starts up.

The cultivation of the tetanus bacillus also furnishes some interesting facts which illustrate the complicated ways in which bacterial growth is 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 bacillus which actively assimilates oxygen, and the two in association will grow in the presence of air.

The tubercle bacillus, when taken direct from an animal, will only grow in a few selected media, such as

blood-serum media, but later on it may be transplanted to agar, and still later to bouillon. After the bacilli have become accustomed to the bouillon they grow with great luxuriance, but only when carefully floated on the surface of the liquid. If submerged in the slightest degree they will not grow.

Many bacteria which demand free access of oxygen grow only in the superficial portion of the nutrient agar jelly, where there is plenty of air.

It is evident, therefore, that for each variety of organism there are special conditions requisite for growth, and that a temperature, degree of acidity, supply of oxygen, immersion in fluid, 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*. Since, therefore, bacteria vary greatly as to the amount of toxin which they produce, and their ability to develop under different conditions outside of the body, we should certainly expect even greater variations in the living bodies of men and animals where not only in different individuals, but even in the same individual at different times, there is a varying suitableness for such growth.

Let us now consider some of the facts which have been observed concerning the growth of bacteria in the body, and then endeavor, as far as possible, to explain them.

In the first place, there are some bacteria which find it impossible to grow in the living body. This is true of the great mass of bacteria occurring in the air, water, and soil. These bacteria cannot, therefore, produce infectious diseases. Some of them, however, produce

poisons in foods, etc., which are absorbable, and which when taken with food or drink can produce a chemical intoxication. That they are really deleterious is shown by the fact that if a sufficient quantity of their pure cultures is injected into the tissues suppuration and abscesses are produced by the toxic substances contained within them.

Closely allied to the bacteria which cannot grow at all in the bodies of warm-blooded animals are those which are able to grow in or upon certain circumscribed areas only. Thus the diphtheria bacilli grow upon the abraded 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 body-surface or in the blood.

Another group of bacteria find, indeed, certain regions most suitable in their conditions for growth, but under circumstances favorable for them are capable of more extensive growth. Thus the typhoid bacillus grows most luxuriantly in the Peyer's 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 at any time may invade many tissues of the body. The gonococcus finds the mucous membrane of the genito-urinary 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.

Still further removed from the saprophytic bacteria are those which grow in the blood and most living tissues as readily as in the most suitable artificial media. Thus a streptococcus which has passed through a number of animals or human beings will, when introduced into the circulation or the tissues, develop as rapidly and generally as in bouillon, and produce death within twenty-four hours, every drop of blood being crowded with bacteria.

Finally, there are bacteria which, in so far as we know, find the bodies of human beings or animals the only fit soil for their growth. These are the true parasites. The leprosy bacillus grows only in man; neither the food nor the conditions suitable for the development of this micro-organism outside of the body have as yet been discovered. The spirillum of relapsing fever is another good example of this group.

Following rather closely the schematic separation of bacteria according to their relation to disease we might classify them as :

1. *Strict saprophytes*, or bacteria which grow readily in suitable dead organic material, but not in the body under ordinary conditions.

- a. Bacteria which in their growth produce no substances which are poisonous to the body, or at least none capable of absorption.

- b. Bacteria which produce in their growth in dead organic matter sufficient poisons to cause sickness if they are absorbed into the animal body.

2. *Facultative Saprophytes*. These are bacteria which can develop either as parasites or saprophytes. The different varieties vary as to the amount of poison which they produce. Some grow luxuriantly in dead

organic material under very diverse conditions, others only under specially favorable conditions. In the body they also vary—some grow extensively in the blood, while others are limited to one or more tissues, some being widely disseminated throughout the body, while others are localized in or upon a certain portion of it.

3. *Strict parasites*, or bacteria which, so far as we know, grow only in the living animal or vegetable organism. These again vary in the amount of poison which they produce and in the local or general infection they give rise to.

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 have a culture of tubercle bacilli, which, after having grown for three years in the bodies of guinea-pigs, will no longer develop on dead organic matter, while a bacillus which was obtained from the same stock, but grown on bouillon for three years, will no longer develop in the animal body. From the same stock, therefore, two varieties have developed, the one being now practically a saprophyte and the other a parasite.

The Local Effects Produced by Bacteria and their Products. Nearly all the forms of acute inflammation are seen to follow the development of bacteria. 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 also 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 products of many bacteria, such as the gonococcus, pneumococcus, streptococcus, and influenza bacillus, etc. The hemorrhagic exudation seen in pneumonia is due to the pneumococcus; it is observed also in anthrax and 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 one species of bacteria produce several forms of inflammation, but the same organism will vary as to the kind or kinds of inflammation it will produce; 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 period of infection, and the circumstances under which the animal remains. Such variations, therefore, are in no case specific, for different poisons will produce changes which appear identical.

The Manner in which Bacteria Produce Disease. The actual mechanical presence of the bacteria is only of importance when, as in septicæmia or pyæmia, they exist

in such enormous numbers 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. These dangerous effects are chiefly due, first, to their alteration of the nutritive substances in the body into others which are valueless, and, second, to their production of substances which are more or less directly poisonous.

A moment's consideration of the different changes which take place in the tissues after the injection of fine sterile sand and of an equal quantity of a dead culture of the tubercle or typhoid bacillus would suffice to convince any one that it was the poison produced by the bacillus, and not its mechanical interference, which caused disease. These poisonous products, as already described in the previous chapter, can be separated from the culture fluid in which the bacteria have grown or they can be extracted from their bodies. These products without the bacteria themselves injected into animals cause essentially the same lesions as are produced by the bacteria when they develop in the animal body. When the body, as a whole, is invaded by bacteria the abstraction from the body of such substances as they consume exerts probably a considerable influence; but even here it is the poisons elaborated by bacteria from the body substances and given up to the blood and tissue cells which are of most importance. The substances contained in or produced by the bacteria, with few exceptions, attract the leucocytes, and when great masses of bacteria die suppuration usually follows.

The General Symptoms Caused by Bacterial Poisons Absorbed into the Circulation. Fever is produced under

favorable conditions by all bacterial poisons. The first requisite is that sufficient poison be absorbed; but, on the other hand, it must not be absorbed with such rapidity as to overwhelm the injected animal, 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.

Centanni¹ obtained through warmth and alcohol from the bodies of bacteria a substance called pyrotoxin, which was with difficulty dialyzed. From different bacteria not only the physiological but also the chemical properties of the pyrotoxin were the same. Not only did this cause fever, but also, when persisted in, it produced emaciation, quickened heart-action, apathy, dyspnoea, etc.

The bacterial poisons produce an increase in the number of leucocytes and a lessening in the amount of hæmoglobin in the blood. 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.

The true bacterial poisons are, as already stated, neither alkaloids nor albumins. Some of them, such as the diphtheria and tetanus toxins, are peculiar in their effects, while others, such as those produced by the pneumococcus and streptococcus, can scarcely be distinguished. They are destroyed by heat at 70° C.

¹ Deutsche med. Wochenschrift, 1894, Nos. 7 and 8.

Bacteria also produce secondary poisons, which stand a temperature of 100° to 120° C.

The Influence of Quantity in Infection. With bacteria the number introduced has an immense influence upon the probability of infection taking place.

If we introduce into a culture medium, which, like the body, is only fairly suitable for growth, a few bacteria, it is not improbable that they may all die; whereas if a greater number are introduced, while there will at first be a slight diminution of these, those that die seem to neutralize the substances which were deleterious; 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 that there will be some surviving members, which, after the destruction of antagonistic substances, and on becoming accustomed to their environment, will begin to grow and produce disease.

With those bacteria whose virulence is great—*i. e.*, those which are capable of growing with great ease in the body fluids—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, a local effect, or in some cases a general septicæmia, by regulating the amount of infection intro-

duced. In the majority of cases in man the number of bacteria received is comparatively small; but by the rupture of an abscess into a cavity or into the circulation, or by the opening of the intestinal contents into the peritoneum, the quantity introduced may be enormous.

The Degree of Virulence Possessed by Bacteria. Bacteria as found in nature differ, as has already been stated, as to the amount of poison they produce and the ease and rapidity with which they grow in any nutritive substance. 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 specific poisons produced by bacteria can be best studied in diphtheria and tetanus. We note, first, that different individual bacilli of diphtheria and tetanus have, when freshly obtained, wide variations in the amount of toxin which they produce—*i.e.*, a diphtheria bacillus obtained from a case of diphtheria will produce in suitable nutrient broth a poison of such strength that 1 c.c. will kill an average sized guinea-pig, while the poison from another bacillus will kill with a much less quantity, or 0.005 c.c. Further, the bacilli obtained from some sources retain their power of producing poison, when grown on artificial media, for years unaltered, while others lose much of this in a few months. This is equally true of the tetanus bacilli.

The power to produce toxin can be taken from bacilli by growing them under adverse circumstances, such as cultivation at the maximum temperature at which they are capable of development. Some bacilli 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 and their growth in capsules placed in the bodies of susceptible animals. 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 suitable cultures, a very minute number will surely develop and produce death. 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 them, and less adapted for all that differ.

Somewhat distinct, again, from that class of bacteria which multiply rapidly are those which, like the tubercle and leprosy bacilli, develop 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 sin-

gle 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 that the virulence in test animals does not usually correspond with the severity of the case from which the organism was derived. We should remember in this connection the varying power of resistance in different animals and of the same individual at different times.

Mixed Infection. The combined effect 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. Mixed infection arises usually from the inoculation of more than one variety of bacteria simultaneously. Thus, an abscess is often due to several forms of pyogenic cocci. If a 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 organism. Thus, whenever two or more varieties of bacteria are transferred to a new soil, mixed infection takes place if more than one variety 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, 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 bacilli or cholera spirilla. Generally speaking, all inflammations of the mucous membranes 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 causing a lowering of the vital resistance of the body.

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 asso-

ciated with them. Again, it is found that the association of one variety with another may increase its virulence. Thus Roux and Yersin believe that they have established the fact that streptococci and diphtheria bacilli mutually increase each other's virulence. 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.

The Modes of Entrance of Infection. The various fluids and tissues of the body differ greatly in their chemical constituents, their reaction, their protection from infection, their access to free oxygen, their temperature, and in other less well-known respects. These variations are sufficient to render certain portions of the body suitable for the growth of some bacteria and unsuitable for others. 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 gives 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.

If we tried in this way all the parasitic bacteria we

would find that certain varieties are capable of developing and thereby producing disease only on the mucous membrane of the throat, others of the intestine, others of the urethra ; some develop only in a wound or in the blood, while others, again, under favorable conditions, seem able to grow in or upon almost any region of the body.

CHAPTER V.

IMMUNITY.

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 is termed natural immunity, in contradistinction to that acquired by recovery from disease.

As in bacteria, we distinguish between the ability to produce poison and the power to multiply in the body, so here we may distinguish between immunity to poison and immunity to the development of bacteria.

With regard to variations in susceptibility, certain known facts have been ascertained. Thus, cold-blooded animals are generally insusceptible to infection from those bacteria which produce disease in warm-blooded animals, and *vice versa*. This is readily explained by the inability of the bacteria which grow at the temperature of warm-blooded animals to thrive at the temperature 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 a micro-organism 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. Differences in susceptibility are sometimes very marked among different varieties of the same race of animals, as, for instance, between different kinds of rats and pigeons to anthrax. In animals, as a whole, it is noticed experimentally that the young of all species 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 hygienic surroundings, exhaustion from overexertion, 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., also give sometimes a point of entrance for infection, or at least a point of less resistance, where the bacteria may develop and produce local inflammation. This is noted in infection by the tubercle and typhoid bacilli, pyogenic cocci, etc. Local affections, such as endocarditis, may also afford a weak spot for the bacteria to seize upon. 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 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.

Immunization and Healing by Non-specific Means. Just as all conditions which are deleterious to the body lessen its power of resistance to bacterial invasion, so all conditions which are favorable to it increase its resistance, and thus aid in preventing and overcoming infection. The internal use of antiseptics against bacteria has not proved successful, for the reason that an amount 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 is, possibly, an exception to the rule, but in both cases we are dealing probably with animal parasites, not ordinary bacteria. Such substances as nuclein and others 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 equal in power to the protective substances formed by the tissues from the bacterial products.

The Use of Local Treatment in Inhibiting Bacterial Invasion. The total extirpation of the infected area by surgical means, if thoroughly carried out, removes the disease entirely; but, unfortunately, this procedure is rarely possible. When incomplete it is frequently helpful; but it may be harmful, for by creating and exposing fresh wounded surfaces to infection it may lead to the further development of the disease. Again, it may be useless, for by removing only a portion of the bacteria it may leave those which have already reached the deeper tissues or blood to go on developing. In some cases, like anthrax and infection from bites of rabid animals, total removal of the virus is possible, either by the knife or thorough cauterization, and will prevent a general infection. 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, incising a cellulitis, or cleansing and drainage of the uterine cavity are well known. The retention of the poisonous products of the bacteria and altered tissue substance leads to their absorption, and thus lowers the tone of the neighboring, and to a less extent of the general, tissues in consequence of the poisoning. This enables the bacteria 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 the bacterial progress. Local bleeding and the application of cold probably act by lessening tension. The application of warmth hastens absorption, and so, when the infection is one which tends to localize, it acts favorably by

accelerating the development and thus the disappearance of the inflammation. A peculiar effect of operative interference is noticed in the frequently observed beneficial result of laparotomy in tubercular peritonitis.

Antiseptic solutions have the power of cleansing and rendering sterile the surfaces of a wound—that is, of preventing 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 as gonorrhœa, diphtheria, and other inflammations 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. Roux has reported that certain specific serums, just as certain enzymes, have some destructive effect on the toxic substances of other species of bacteria; but this is a subject which has been as yet but little investigated.

Specific immunity, or a condition of the body which prevents the development in it of one variety of micro-organisms and renders it unaffected by their bacterial poisons. The invasion of the body with more or less serious results by most micro-organisms is followed by a condition which for a variable period and to a

variable degree is deleterious to their further growth. It also gives rise to substances which neutralize the poisonous effects of the bacterial products. This immunity may take place in various ways :

1. Through recovery from disease naturally contracted or from infection artificially produced. This immunity may be slight, as after recovery from erysipelas or pneumonia, marked for a short period of time, as in diphtheria and typhoid fever, or prolonged, as after scarlet fever or syphilis.

2. By the injection of the bacteria into tissues not well suited to their development, as the injection of typhoid bacilli or cholera spirilla into the subcutaneous tissues. Here a mild local infection follows, with considerable resulting immunity.

3. By the injection of micro-organisms attenuated by heat, chemicals, or other means. In this case a local or general infection of the animal is produced, of moderate severity, as a rule, and the immunity is not 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 in man are examples of this method.

4. By the injection of the unaltered chemical constituents of the dead bodies of bacteria and of the chemical products which they elaborate and discharge into the surrounding culture media during life. Smith and Salmon proved that by repeated injections of the filtered bouillon cultures of the hog-cholera bacillus a considerable immunity may be produced against the invasion of this bacillus. Similar results have followed the injections of dead cultures of typhoid and anthrax

bacilli and cholera spirilla, etc. After infection with most parasitic bacteria the body resistance to the growth of the same organism is greatly increased; in other infections, however, it is but slightly augmented.

The protective substances held in solution in the blood-serum are clearly apparent in their effects either in preventing the increase of the bacteria or neutralizing the toxic action of their products; chemically, however, they are but little understood, and although some of them have been shown to be to a large extent specific, that is, they are far more efficient in protecting against the special variety of bacteria which produced the infection than against any other, still we have no knowledge of any chemical difference between them. The addition of 0.5 per cent. of carbolic acid injures these substances but slightly. At ordinary temperatures there is a gradual deterioration in value, so that in from one to six months they may become inert. Twenty hours' exposure to a temperature of 60° C. does not destroy them, but one hour at 70° C. does so almost totally. Different protective substances differ as to the rapidity with which they deteriorate.

Suitable animals after repeated infections gradually accumulate in their blood considerable amounts of these protective substances, so that very small amounts of serum 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 one hundred 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 all cells to a greater or less extent. How much of this is simply in solution from the serum, and where the substances are formed, is not definitely known.

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. Héricourt, Richet, and others demonstrated the same thing for other diseases. The results obtained by Behring and Kitasato upon diphtheria and tetanus, where, indeed, the serum prevented the action of the poisons rather than the direct development of the bacteria, gave a still greater impetus to these investigations.

The immunity produced by these substances affects the entire body, as is only natural, since the blood into which they are absorbed is distributed everywhere. When the immunity is but slight, infection may take place in the more sensitive regions and still be impossible in those tissues having more natural resistance. If the serum is injected into other animals or man the immunity is greatest immediately after absorption, and then declines, being rather quickly (in several weeks or months), almost entirely lost, so that repeated injections are required to maintain the immunity. This is distinctly in contrast to the immunity acquired after the introduction of bacterial products, where the tissues

of the organism, in ways unknown, give out, in response to the bacterial stimulus, inhibitory or antitoxic substances, or combine with the bacterial poisons to produce them. Here immunity reaches its height a week or ten days after the injection, and then continues for a week or two, when it slowly declines again. The serum immunity is frequently called passive immunity and the bacterial immunity active immunity.

If a greater quantity of protective substance is desired in the blood than occurs after one infection, repeated injections of living or dead bacteria and their products are given, the doses being administered at short intervals and in sufficient amount to produce a slight elevation of temperature and malaise. Then, as soon as the animal returns to a normal condition, another injection of slightly greater quantity is given. After several months of such treatment the blood is withdrawn, allowed to clot, and the serum then siphoned off aseptically and stored either with or without the addition of preservatives. 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 bacteria 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 micro-organism will be neutralized by several times the amount of serum which a single fatal dose requires, since in the case of very virulent living 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. 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, but some serums will prevent further development even then. The immunity conferred on a person from serum lasts from a few days to several months, according to the amount of serum injected. As in animals, it is strongest immediately after absorption. An injection of bacterial poisons or the contraction of actual disease usually confers immunity from one to three weeks after the infection, and lasts, according to the nature of the infection, from one month to a year or more. The serum loses all appreciable protective value as measured in test animals in the usual doses before the person is liable to infection. Repeated injections of serum continue this condition of immunity indefinitely.

The use of serums having specific protective properties has been tried both in animals and man as a preventive of infection. In susceptible animals injections of some of the very virulent bacteria, as pneumococci, streptococci, typhoid bacilli, and cholera spirilla, 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 ineffective. For some bacteria, such as tubercle bacilli, no serum has been obtained of suffi-

cient power to prevent infection. Through serums, therefore, we can immunize against an infection, and even stop one 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 have not given, as observed in practice, conclusive evidence of great value in already developed disease. This is partly due to the difficulty to be discussed fully later of determining early enough the exact nature of the bacteria causing the infection.

Acquired Immunity to Poison. 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 of these protective substances may be 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, and these doses in the more suitable animals can be gradually increased until a thousand times a previously fatal dose may be administered without any serious results whatever. 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 toxin.

Some other important parasitic bacteria produce toxins and in the body antitoxins, but all to a far less extent than those of tetanus and diphtheria. Following them is the plague bacillus, and then, but far behind, the cholera spirilla, the typhoid bacilli, the streptococci, etc. These latter bacteria produce more of the substances which inhibit bacterial growth than of those which neutralize their toxins.

The effect of the antitoxin is to prevent the poisonous action of the toxin. It does not, so far as we know, influence the cells after they have been injured by the toxin; it is, therefore, 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 profoundly poisoned by the toxin is unaffected by any amount of antitoxin.

The antitoxins of diphtheria and tetanus are gradually eliminated from the body after their injection or after their production from toxin injections. After the usual immunizing dose the duration of immunity is only from two to six weeks, the period differing in each individual. The elimination of the antitoxin takes place partly through the urine and other secretions, and it is partly destroyed in the body. An animal which has been highly immunized will retain considerable amounts of antitoxin for from two to four months.

The antitoxins as contained in the serum are fairly stable. The different antitoxins vary thus, that of diphtheria is somewhat more stable than that of tetanus. Kept aseptically in cold and dark storage, and pro-

tected from access of air, the more resistant antitoxins may be preserved sometimes for a year or two with practically no deterioration in strength. At other times, however, from unknown causes, they are gradually destroyed, so that there may be a loss of about 10 per cent. per month. A serum requires, therefore, to be tested every few months if we wish to be assured of its strength in antitoxin. Preservatives, such as carbolic acid, trikresol, camphor, 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. In animals injected with diphtheria toxin Atkinson has found that there is with the increase in antitoxin an almost proportional increase in globulin.¹ He also found that the antitoxins behave like globulins with the various reagents, being completely precipitated by magnesium sulphate. NaCl, when added to saturation to globulin solutions holding antitoxin, partially precipitates the globulin and the antitoxin. When raised to 72° C. a series of precipitations are obtained which contain at least the greater part of the antitoxin. Whether this indicates that antitoxin is a form of globulin or merely that it is similarly affected by many reagents, and that the toxin in some way stimulates the development of both, it is as yet impossible to say. Although in a very rough way, the same animal produces antitoxin in direct proportion to the amount of toxin injected so long as its condition remains good, yet different animals of the same species give very varying amounts from the same injections, some not giving one-

¹ This work, carried out in the Research Laboratory of the Department of Health of New York City, will appear in the *Journ. of Exp. Med.* in 1900.

fourth of that furnished by others. The antitoxin produced by a certain number of fatal doses of toxin will neutralize many thousand times that amount.

Diphtheria and tetanus antitoxin are measured by the protective power of the serum in which they are in solution—that is, the amount of serum required to protect susceptible animals from a certain number of fatal doses. In diphtheria this is measured in units; in tetanus usually by the proportion which exists between the amount of serum used and the animal's weight. For detailed information, see under Diphtheria and Tetanus.

Antitoxins are absorbed to a very slight extent only when taken by the mouth—certainly less than 5 per cent. They must, therefore, be introduced subcutaneously or intravenously to enter the body. The antitoxic serum does not act against the bacteria directly, but by neutralizing their poison, it prevents them from acting as irritants to the cells, and so the soil for the growth of the bacteria becomes unsuitable, and they cease to develop. The diphtheria bacilli grow perfectly well in their antitoxic serum.

The Elimination of the Bacteria and Their Products. This takes place by the direct separation and removal of the bacteria where there is access to the outside, such as exists in the mucous membranes of the respiratory, digestive, and urinary tracts, and from the cutaneous surfaces, etc. The elimination of the bacteria and their products is almost a necessity where there has been any great accumulation, if healing occurs. When the bacteria have penetrated deeply into the tissues, and continue steadily advancing, the elimination from the surface is of little curative value, as the number thrown off is so small in comparison

with the number remaining. Occasionally the casting off of the bacteria allows them to infect other places, as in some cases where laryngeal and intestinal tuberculosis follows pulmonary tuberculosis. We must bear in mind, however, that infection in these regions may have been produced through the lymph and blood channels.

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 stomata of the capillary walls; (2) carriage into the blood in the bodies of leucocytes; (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 bloodvessels.

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, by means of the leucocytes, which penetrate the capillary walls, or, directly, they pass into the tissues and substance of the organs. They thus reach the lymph channels and glands, or through the secretions 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 excretions into the urine, as in typhoid fever, though some deny that this can happen unless there is a previous inflammation of the kidneys. The passage of bacteria through the breast is important, from the fact that milk is so largely used as food. Many observers have reported

the finding of tubercle bacilli in milk when the gland itself was intact and the animal tubercular. 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, and probably these observers have been deceived by the pseudo-tubercle bacilli. They are undoubtedly present, however, in the milk of some animals in which tubercular disease of the gland could not be demonstrated. 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 usually streptococci, which have often been received from the mouth of the sucking infant.

Whether bacteria are eliminated 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. So long as the organs of secretion are not injured it is not likely that many micro-organisms are eliminated from the blood in this way. 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. Such removal, moreover, has little if any beneficial effect; but, on the other hand, may be a source of danger to others, as in typhoid fever. The removal of the poisonous products of bacteria by the kidneys, intestines, etc., on the contrary, is of great advantage to the organism.

CHAPTER VI.

THEORIES OF INFECTION, IMMUNITY, AND RECOVERY.

THE tissues of the animal body under the normal conditions of life are, as we have seen, unsuitable for the growth of the great majority of the varieties of bacteria. Indeed, only a very small number of the parasitic bacteria find the conditions really satisfactory, and even these must find a point of entrance into the body.

In seeking to account for the difficulty which to a greater or less extent all bacteria find to growing in the tissues of the living body, we cannot find it either in the insufficient or excessive concentration of the nutritive substances, nor in the temperature, nor in the reaction; for although some of these conditions may be unsuitable for some bacteria, they are all suitable for many, and thus cannot constitute the fundamental explanation of either natural or acquired immunity. A possible ground, for the inability of the bacteria to invade living tissues, might be thought to be found in the fact that the nutritious material in the living cells is in a form which the bacteria cannot readily assimilate; but, if this be true to a certain extent, it does not adequately explain why the bacteria do not develop in the nutritious fluids, so abundant about and in the body tissues, nor does it account for acquired immunity. We are thus driven to the conclusion that the body fluids themselves contain substances which are directly dele-

terious to the bacteria. As to the origin of these substances, we may conceive that they may be either regularly produced by the body cells, or by the fluids, or by both, or that they may only be produced or at least increased when bacteria invade the body. When formed they may remain unaltered in the fluids or be quickly eliminated or destroyed. It is probable that more than one of these suppositions is actually true.

The bactericidal effect upon most bacteria of the body fluids, noted by Nuttall in 1888, is now undisputed, and is shown by the fact that bacteria when injected into the blood usually soon die, and this destruction may be so rapid that in a few hours none remain alive. Even when bacteria survive and produce infection there is for a time a decrease in the number living, but this is soon followed by a progressive increase. This fact can be observed not only by injecting bacteria into the blood and peritoneal cavity, but also when the bacteria are placed in the animal body after being enclosed in capsules. The bacteria are killed even if they have previously grown outside the body in blood-serum. Bacteria have also been injected into a vein carefully ligated above and below, and here, without coagulation, the blood exerts bactericidal properties. The general germicidal effect of the blood-serum can also be watched outside of the body. Here mixed with it some species of bacteria die quickly, some slowly, and some lose only a portion of their number, those remaining alive after a time rapidly increasing. The number of bacteria introduced is of great importance, for the serum with its contained substances seems capable of destroying only a certain number, and after that loses its bactericidal properties.

If the bactericidal effect of the serum outside the body always went hand-in-hand with the immunity of the individual from which it was taken, the immediate cause of immunity would be solved and our search be directed to find the source and nature of these germicidal substances; but this is not wholly the case, for while in many instances it is so, in others it is as undoubtedly not true. We must, therefore, add to the serum the activity of the cells, which produce constantly the substances which are partly given up to the blood and fluids of the body and partly retained in their own bodies. This deleterious action of the blood in bacteria can be increased by infection. Some good observers have found that blood in animals naturally immune to certain parasitic bacteria, which had little or no bactericidal effect, became possessed of it after a moderate infection; this seeming to indicate a protective effort of the body cells to withstand bacterial invasion.

Concerning the nature of these non-specific protective substances, named *alexines* by Buchner, we have as yet little positive knowledge, but certain properties of them are known. They are largely precipitated by a 40 per cent. solution of sodium sulphate, but not by alcohol. These substances would seem to belong to the so-called living proteids, and resemble certain of the globulins in their properties, but they are evidently extremely complex in their nature. Many of them become inert on standing for several months, even at low temperatures, and after a few weeks at blood-heat. A temperature above 62° to 70° C. soon totally destroys them. Freezing does not affect them. A bactericidal serum affects in a deleterious manner the red blood-cells of a different species of animals.

Their source must apparently be attributed to the cells, but probably certain cells only produce them. The red blood-cells, for instance, seem rather to destroy than to increase them. The nuclein derived from the cells, although it has a general bactericidal action, and may enter into the alexines, yet as it has different properties it cannot itself be one of these bodies. The cells which have abundant nuclear substance, such as the leucocytes and lymph-cells, seem especially to be a source of the alexines. Buchner and others have found that through the irritation of bacterial filtrates the leucocytes were attracted in great numbers to the region of injection, and that the fluid here, which was rich in leucocytes, was more bactericidal than that of the blood-serum elsewhere. The same fluid acted also more perfectly when it contained numbers of leucocytes than when they were filtered off. Substances similar to the alexines are apparently derived from the leucocytes, and their attraction to the injected area gives to that location greater protective power. Some claim to have demonstrated that along with increased leucocytosis there is a general increase in the alexines in the blood, still it has not yet been positively established that the alexines are derived solely from the leucocytes, nor from all leucocytes, and a mere increase in them does not always mean an increase in alexines. The attraction between the leucocytes and the bacteria is due to the chemical attraction between them and the bacterial body substance and its poisons. Some chemical substances not derived from bacteria have this quality also, called positive chemotaxis, while others repel the leucocytes—negative chemotaxis. The original theory of Metchnikoff, that the leucocytes were the only actual

protective bodies which warded off disease, and that they did this by attacking the bacteria, was founded on the observation that certain of the white cells possessed the power of taking up into themselves pathogenic bacteria, and that they were there destroyed. It was later observed that these cells had 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 influences killed or injured them before the cells took them up. The theory then became somewhat modified, more knowledge was obtained, and it is now believed that the bacterial substances attract the cells, and that when these cells are brought together the general, and perhaps the specific, bactericidal property of the blood in their neighborhood is thereby increased. The death of the bacteria liberates this positive chemotaxic substance, and the disintegration of the white blood-cells gives rise to the bactericidal bodies. Thus we find that phagocytosis is most marked when the disease is on the decline or the infection mild, but that in rapidly increasing progressive infection it is absent. This would seem to indicate that the course of the infection is often already determined before the leucocytes become massed at the point of its entrance. The first determining influence is given by the condition of the tissues and the bactericidal substances contained in them, and then, later, in cases where the infection is checked, comes the additional bactericidal substance given off by the attracted leucocytes. In so far as the tissues themselves are unsuitable for the development of bacteria they are sufficient to ward off infection, but

in proportion as they are incapable of doing this they are assisted by the substances contained in the leucocytes. If the tissues are wholly adapted for the growth of the bacteria, neither they nor the leucocytes, nor both combined, can furnish sufficient protective substances to prevent the bacterial increase. The entrance of bacteria into the leucocytes, which is not infrequent, may mean their destruction; but, on the other hand, the bacteria may increase in the white blood-cells and destroy them, and they may be killed without entering the cells. The simple absorption by the cells is not necessarily a destructive process. No explanation can as yet be given of natural immunity to bacterial poisons, except that it may be connected with some general property of the tissue. There is far less variation among different species in their resistance to the bacterial poisons than in their suitability for the growth of the living bacteria which produce them. Possibly certain organs, such as those which are rich in nuclein—for example, the lymph-glands, the liver, etc.—may have some destructive power with regard to poisons. The nature of the cell substance is known to have much to do with its relations to certain poisons. Thus the tetanus poison acts chiefly on the nerve cells and leaves the others almost or altogether unaffected.

By what means are virulent bacteria enabled to increase in the body, notwithstanding its protective powers, when non-virulent organisms of the same species are incapable of so doing? This is but little understood, but experiment shows in the first place that both virulent and non-virulent forms are equally resistant to general destructive agencies; and, second, that the bacteria are capable of producing substances (*lysines*) which

neutralize in some way the protective substances (alexines). The virulence of bacteria would, therefore, depend partly upon their ability to produce these lysines, which act perhaps as the ferments upon the alexines, or perhaps combine with them. That bacteria under certain conditions form specific poisons, and under others, even when they grow luxuriantly, do not, is clearly shown by our experiments on the production of diphtheria toxin. Here, as previously stated, it was found that when the bouillon was either a little too alkaline or too acid, though the bacilli grew rapidly, they did not produce specific toxins. By growing the bacilli for a time in such bouillon they eventually became able to develop toxin in a soil in which they previously failed to do so. Similar cultivation in the body may be assumed to increase their ability to produce specific poison after a while under what would at first be adverse conditions.

With regard to the increase and decrease of general, and perhaps also of specific immunity, we have reason to believe that as the protective substances are produced by the living cells, anything which lowers the general vitality must lessen the vitality of the cells, and thus their ability to produce protective substances in the amount possible in a normal condition. The attraction of leucocytes to any point by some new infection might increase the germicidal action of the tissues, and so influence the first infection.

Specific Immunity. The following theories have been advanced concerning the nature of specific immunity: The theory that a second infection is impossible because the first used up substances which were necessary to the growth of the bacteria is untenable for many reasons. Thus it can be demonstrated that the injection of a

small amount of specific serum, which robs the tissues of nothing, produces the same immunity. Again, the injection into the body of a sufficient number of pathogenic bacteria gives rise to an infection in all cases.

The theory of Metchnikoff, that the leucocytes or wandering cells of the body, after an infection with a certain variety of bacteria, become influenced in some way, so that they attack especially that form of infection again and destroy the bacteria (phagocytosis), can no longer be considered as more than a very partial explanation, and can only be accepted by assuming as proven a number of hypotheses.

The retention theory of Wernich and Chaveau, somewhat modified, has much to support it. The blood-serum of animals recovering from an infection was found to have changed chemically to such an extent as to be capable of being demonstrated experimentally, and these changes were shown to persist for a number of weeks or months or even years. Similarly the serum of immunized animals retains for a long time its immunizing substances. We are, therefore, compelled to accept the fact, that when an infection is passed through there are more or less protective chemical substances left in the blood, which remain there for a considerable time. Kruse believes that these substances have the power of neutralizing the bacterial poisons which are given off by the bacteria upon their entrance into the body, and of thus robbing them of their deleterious effects on the alexines; the body fluids in this way remaining unsuitable soil for the growth of the bacteria, the alexines being bactericidal. If only a small amount of antilynsines are present some of the alexines are destroyed and the bacteria are not all killed or

weakened. Those remaining active are then further acted upon by the alexines in the tissues and by the substances given off by the leucocytes. If these protective substances are insufficient the infection is established. R. Pfeiffer's experiments with cholera and typhoid cultures injected into the peritoneal cavity of the guinea-pig along with specific protective serum showed that the bacteria were altered and destroyed by the serum within a few minutes, just as if they had been non-virulent bacteria, and this without the assistance of the phagocytes. In this case non-virulent bacteria die because they produce no lysines to destroy the alexines, while those which are virulent do not thrive, because although they produce lysines, the antilynsines in the serum destroy them, being thus acted upon by alexines in like manner to the non-virulent bacteria.

As to the development of the specific protective substances, the most plausible theory seems to us to be that they are formed by the activity of the cells from the bacterial poisons, the lysines. These substances are stated by Pfeiffer and Marx to be most abundant in the spleen, lymphatic glands, and bone-marrow.

Ehrlich and others believe antitoxin to be a portion of the substance of certain cells, which, having been stimulated by their effort to replace portions of their substances destroyed by previous doses of toxin, have reproduced it in excess. This cell substance, being free in the fluids of the body, combines with the toxin, and thus neutralizes it. But it is difficult by this theory to explain many known facts, such as the one that a fully neutralized mixture of toxin and antitoxin is still capable of producing in the body more antitoxin. Others hold that the antitoxins, as the other

protective substances, are always present in the body, and that under special need, as when bacterial invasion takes place, they are thrown out by the cells in larger quantities, and this is especially true of the special substances needed for existing infection. The antitoxin then acts by fortifying the cells so that they are enabled to resist the action of the toxin. In favor of this view is the fact that the cells of certain animals are undoubtedly proof against these toxins, and yet so far as chemistry in its present development can detect, these cells are the same as similar but sensitive cells in other animals. Another theory is that the toxin, in some way in the body fluids, is changed into antitoxin. This is made slightly plausible by the fact that by the action of electricity there have been obtained substances from toxins which are slightly antitoxic. The practical point to remember is that whether or not the theories are correct, there is no doubt that the protective substances exist.

CHAPTER VII.

INFECTION.

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 thrown off from the body and the prevalence of the disease.

In diphtheria, typhoid fever, cholera, pulmonary tuberculosis, septic endometritis, influenza, and gonorrhœa enormous numbers of infectious bacteria are cast off through the discharges from the mouth, intestines, and genito-urinary secretions, causing great danger of infection. On the other hand, in tubercular peritonitis, cerebro-spinal meningitis, septic 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, and leprosy bacilli do not develop, as far as we know, outside of the body under ordinary conditions. Others, like the diphtheria bacillus and the streptococcus, may under certain conditions, as in milk in warm places, develop and produce infection. Others, again, such as the streptococcus and staphylococcus, typhoid and anthrax bacillus, the cholera spirillum, and some anaërobics, 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 anaërobic bacteria to permit of their development in the soil in the presence of oxygen.

A large number of the infectious bacteria are able to develop in or upon some portion of the skin or mucous membrane, either after or before disease, and without

causing infection. As complete a knowledge of these facts as possible 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 the 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 one hundred throats examined by the writer in New York City, streptococci and staphylococci could be found in over 90 per cent., and pneumococci were very frequently discovered. Many other varieties of bacteria, such as 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 exposure to cold or injury of any kind, owing to the presence of these bacteria, the persons harboring them may develop tonsillitis, tonsillar abscess, or diphtheria; or the bacteria may invade the bronchial mucous membrane or the lungs. The diphtheria bacilli, and perhaps other bacteria, transmitted to others may become the source of infection to them, though the person who spreads the infection may remain unaffected.

The stomach, on account of the acidity of its contents, is comparatively free from bacteria. The normal

intestines, on the other hand, contain great numbers of bacteria. Among these the colon bacillus is constantly present, and often the streptococcus and other pathogenic bacteria. After typhoid fever the bacilli may remain in the intestinal contents for weeks and in the bladder and gall-bladder for months. The bacteria swallowed to a considerable extent escape destruction in the stomach, and thus appear in the intestines. Some good observers have stated that bacteria can be absorbed through the intestinal wall into the chyle and blood. When the intestinal canal is injured, or its circulation hindered by strangulation, etc., the 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.

The kidneys, bladder, and urethra may be the source of infection and may give rise to disease in others. 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. After typhoid fever the urine may contain abundant typhoid bacilli for weeks and be little thought of as a source of infection. A persistent gonorrhœal vaginal infection may lead to a gonorrhœal endometritis, or salpingitis, or peritonitis, under suitable conditions. The staphylococci in the skin and the colon bacilli and pyogenic cocci in the fæcal discharges may also be carried into the uterus and produce septic infection.

Inherited Infection and Susceptibility to or Immunity from Infection. The passage of bacteria from the mother's blood through the placenta to the fœtus has been demonstrated for numerous bacteria, among the most important of which may be mentioned the pneumococcus, streptococcus, and tubercle bacillus. The detection of the tubercle bacillus by Gärtner and others under these circumstances prevents us from denying the possibility that tuberculosis developing in children may have been due to infection taking place before birth. The fact, however, that calves removed from tuberculous cattle and fed on milk free from tubercle bacilli do not develop tuberculosis, while those left with tuberculous cattle become tuberculous, indicates that tuberculosis in man also is usually, at least, due to infection after birth. The infection from spermatozoa is conceivably possible in tuberculosis if the testicles are affected; the same may be said of syphilis; but except for syphilis, in which the nature of the infective agent is unknown, we believe that such infection is, if ever present, extremely rare.

Natural immunity pertains more to species than individuals, and such immunity is handed down by the parents to their offspring. If the immunity of one or both parents has been acquired by them during their lifetime previous to the birth of the offspring the immunity conferred is slight or none at all. This is especially true of the male side. In the case of the female parent another factor comes into play after the fructification of the ovum—viz., the absorption of products from the fluids of the mother, for the placenta is no barrier to soluble substances. Thus, sheep which have been immunized to anthrax have moderately immune

young. On the other hand, animals vaccinated with cowpox have not been found to have immune offspring. Toxins injected into the parents apparently do not pass the placenta; but antitoxins do, giving thus a slight transitory passive immunity. A slight immunity is also given by immune mothers through their milk, a small amount of antitoxic substance being absorbed.

CHAPTER VIII.

THE EFFECT OF LIGHT, ELECTRICITY, PRESSURE, AGITATION, DRYING, AND ASSOCIATION WITH OTHER MICRO-ORGANISMS UPON BACTERIA.

VERY little is known about the 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 influence on the culture liquid may produce changes which finally sterilize it. Gottstein and Spilker have recently made experiments with an induction current from a dynamo machine. They passed the current through a spiral wire, which was wrapped around a test-tube of glass containing the micro-organisms to be tested, suspended in water. The bacillus prodigiosus, suspended in sterilized distilled water and contained in test-tubes having a capacity of 250 c.c., was subjected to a current of 2.5 ampères + 1.25 volts for twenty-four hours. The temperature did not go above 30° C. No growth occurred when the organism tested was subsequently planted in nutrient gelatin. It was found that stronger currents were effective in a shorter time, but in no case was sterilization effected in less than an hour. These experiments, however, have not been confirmed.

Meltzer has shown that while slight agitation of cultures of bacteria acts favorably on their develop-

ment, the vitality of bacteria is destroyed by protracted and violent shaking, which causes a molecular disintegration of the cells.

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.

Influence of Light. A large number—perhaps the majority—of bacteria are inhibited in growth by the action of diffuse daylight, still more by that of direct sunlight, and when the action 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 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 shut off by allowing the ray of light to pass first 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 a sharply defined region of the shape of the paper strip crowded with colonies lying in a clear sterile field.

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 vitality in from five to six hours. 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.

Only the ultraviolet, violet, and blue rays of the spectrum seem to possess bactericidal action; green light is very much less so, red and yellow light not 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 least 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 One Species upon the Growth of Another. While it is the custom of bacteriologists to have pure cultures to work with, we should never forget that in nature bacteria often occur in mixed cultures. If we examine water, milk, or the contents of the intestines of either sick or healthy persons we shall always find several species of bacteria occurring together. This admixture may, perhaps, seem to us at first merely accidental, but on further investigation it will appear that also in the department of bacteriology there exist synergists and antagonists, or at least bacteria which assist or oppose one another mutually or one-sidedly. Nencki speaks of *symbiosis* and *enantobiosis*.

Gassé has demonstrated experimentally the existence of antagonisms by inoculating gelatin streak cultures of various bacteria; it is found that many species will not grow at all or only sparingly when in close proximity to some other species. This antagonism, however, is often only one-sided in character; for instance, the

bacillus fluorescens putidus grows well when inoculated between streaks of *staphylococcus*, but the latter micrococcus will not grow at all when inoculated between cultures of the *bacillus putidus*, the growth of the *staphylococcus* remaining scanty when the two species are inoculated simultaneously. Again, when gelatin or agar plates are made from two different species of bacteria inoculated into the same tubes while liquid it may be observed that only one of the two grows. A third method of making this experiment is to simultaneously inoculate the same liquid medium with two species, and then examine them later, both microscopically and in thin plates; not infrequently the one species may take precedence of the other, which it finally overcomes entirely. The practical application of this is to make only very thin plates for the estimation of the number of bacteria or the isolation of pure cultures.

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

The symbiotic or co-operative action of bacteria is of still greater importance, of which the following examples may be given:

1. Some bacteria thrive better in association with other species than alone. Certain anaërobic species grow even with the admission of air if only other aërobic species are present (tetanus).

2. 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.

3. In like manner it is observed that in a series of

soil bacteria none by itself may be pathogenic, but when inoculated into animals in certain combinations produce disease.

4. Slightly pathogenic species, such as attenuated tetanus bacilli, for example, gain in virulence when cultivated together with the proteus vulgaris.

Want of Nutrition and 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 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. Want of water affects bacteria 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 it. A shrunk 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. The following table of the results obtained by Sirena and Alessi 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 "	1 "	5 days	59 "
B. typhosus	41 "	1 "	18 days	64 "	68 "
B. mallei	35 "	44 days	31 "		
Diplococ. pneumoniae .	114 "	31 "	131 "	164 "	192 "

The spirillum of Asiatic cholera is notorious for its slight resistance to desiccation; according to the exhaustive investigations of Koch and the above-mentioned observers, its life-duration is only from one to five hours, depending upon the method of desiccation employed.

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 Berckholz and 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.

Behavior Toward Oxygen and Other Gases. As already noted under the nutritious substances required by bacteria, it is customary to divide bacteria into three classes, according to their behavior toward oxygen.

1. **Aerobic Bacteria.** Growth only in the presence of oxygen; the slightest restriction of air inhibits development. Spore-formation especially requires the free admission of air.

2. **Anaerobic Bacteria.** Growth and spore-formation only in the total exclusion of oxygen. 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; these spores, on the contrary, are very resistant. Anaërobic bacteria being deprived of oxygen—the chief source of energy supplied to the aërobic species, by which they oxidize the nutritive substances in the culture media—they are dependent for their nutrition upon decomposable substances, such as grape-sugar, which on separating into two smaller molecules—alcohol and carbonic acid—give out energy or heat. Anaërobic bacteria, therefore, require for their cultivation, as a rule, media containing 1 to 2 per cent. of glucose or some equivalent.

3. **Facultative Aerobic and Facultative Anaerobic 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 in the partial exclusion of oxygen. Life in the animal body, for example, as in the intestines, necessitates existence with diminished supply of oxygen. Pigment formation almost always ceases with the exclusion of oxygen, but poisonous products of decomposition are more abundantly produced (Hueppe).

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 showed 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 for anaërobic growth is not sufficient for its separation into a distinct species.

While all facultative 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 offer a great resistance, rendering impossible complete sterilization by means of this gas. Another group, again—viz., streptococcus and staphylococcus—exhibits

a scanty growth; while a third group, like the *B. typhosus* and *B. prodigiosus*, are not at all affected, growing equally well as in the presence of oxygen, and the liquefaction, even of gelatin, not being interfered with; only, on account of the lack of oxygen, there is no pigment formation. Finally, 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 .

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

CHAPTER IX.

EFFECT OF TEMPERATURE UPON BACTERIA.

IN judging the effect on bacteria of different agents we have first to note the important fact that different species of bacteria are differently influenced by the same substances. Some bacteria thrive under conditions which would destroy others, and they vary among themselves in their powers of resistance to influences which are deleterious to all.

Further, any species of bacteria will resist better when under favorable conditions than under unfavorable ones. Bacteria also in recent cultures withstand injury better than those in old cultures, so long as they have not entered into the spore form. According to the amount of injury they have suffered, bacteria may be only lamed in some of their functions or they may be totally destroyed. Their loss of function may be only temporary or permanent.

Every bacterial species makes certain demands on the temperature of its culture media. Vegetative life is possible within the limits of 0° and 70° C. There are some species, however, which grow at the lower and others at the upper limit of these temperatures. The maximum and minimum temperature for each individual species lies about 30° C. apart. 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 the water bacteria, such as the phosphorescent bacteria in sea-water.

Mesophilic Bacteria. Minimum at 10° to 15° C., optimum at 37° C., maximum at about 45° C. To this class belong all pathogenic bacteria, the conditions for their pathogenic action in man requiring acclimatization to the temperature of the body.

Thermophilic Bacteria. Minimum at 40° to 49° C., optimum at 50° to 55° C., maximum at 60° to 70° C. This class includes many soil bacteria and almost exclusively spore-bearing bacilli. According to Globig, there are about thirty species of bacteria capable of development at 60° C. and a few at 70° C. Miguel has described a certain *bacillus thermophilus*, which thrives at from 42° to 72° C., its optimum being at 65° to 70° C., and found in cloacæ, the contents of the intestines, and in dirty water. Rabinowitsch has recently described eight thermophilic facultative anaërobic species, all spore-bearing, non-motile bacilli, the optimum temperature of which is from 60° to 70° C., though they grow slowly at from 34° to 44° C., and best on anaërobic agar cultures. They are found widely distributed in the feces.

By carefully elevating or reducing the temperature Dieudonné has succeeded in increasing the limits within which a variety of bacteria will grow. Thus, anthrax was gradually made to accommodate itself to a temperature of 42° C., and pigeons, which are comparatively immune to anthrax, on account of their high body temperature (42° C.), when inoculated with this anthrax succumbed to the infection. Dieudonné also

gradually acclimated anthrax to a temperature of 12° C., when it 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.

Bacterial growth is retarded by temperatures only a little below the minimum of the species in question; but they are not otherwise injured. Indeed, it is the usual custom in laboratories to preserve bacteria which die readily (such as streptococci) by keeping them in the refrigerator at about 4° to 6° C., after cultivation for two days at 20° 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. Ordinarily, low temperatures, though arresting the growth, do not destroy the vitality of bacteria. This has been demonstrated by numerous experiments in which they have been exposed for hours in a refrigerating mixture at -18° C. They have even been subjected by us to a temperature of -175° C. by immersing them in liquid air kept in an open tube for two hours, and found to grow still when placed in favorable conditions.

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 80° and 100° has the same result as a shorter one at the higher temperatures. According to Sternberg, ten minutes' exposure to moist heat will at 52° C. kill the cholera spirillum, at 54° C. kill the streptococcus, at 56° C. the typhoid bacillus, at 60° C. the gonococcus, and at 62° C. the staphylococcus, the latter being about the most resistant of the pathogenic organisms which have no spores.

When micro-organisms 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 to resist a temperature of over 100° C. dry heat for an hour. A temperature of 120° to 130° C. maintained for one and a half hours is required to destroy all bacteria, in the absence of spores, if dry heat is used.

Spores are far more resistant to all injurious influences than vegetative forms. They retain their power of germination for years without either nourishment or water, and are much more indifferent to the action of gases than bacilli, the spores of the anaërobic species being especially resistant to the action of oxygen. 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. The spores of bacillus anthracis and of

bacillus subtilis require a temperature of 140° C. (dry heat) maintained for three hours to insure their destruction. Moist heat at a temperature of 100° C., either boiling water or free-flowing steam, destroys the spores of known pathogenic bacteria within ten minutes; certain non-pathogenic species, however, resist this temperature for hours. Thus, Globig obtained a *bacillus* from the soil, the spores of which required five and a half to six hours' exposure to streaming steam for their destruction. These spores survived exposure for three-quarters of an hour in steam under pressure at from 109° to 113° C. 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 resistance of spores to moist heat is tested by suspending cover-glasses upon which the spores (anthrax) have been dried in little gauze bags in a boiling steam sterilizer. The cover-glasses are removed from minute to minute and laid upon agar plates, which are then placed in the incubator at 37° C. Anthrax spores are obtained by carefully removing sporulating streak cultures on agar and heating the emulsion prepared with a little water to 70° C. for five minutes.

In the practical application of steam for disinfecting purposes it must be remembered that while steam under pressure is more effective than streaming steam it is scarcely necessary to give it the preference, in view of the fact that all known pathogenic bacteria and their spores 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" it has about the same germi-

cidal 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. 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 hot-air oven at 133° C. and over.

Fractional Sterilization (Tyndalization). Certain nutrient media, such as blood-serum and the transudates of the body cavities, as well as certain fluid food-stuffs, such as milk, 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 55° to 70° C. for one hour on each of six consecutive days. By this means we kill, upon each exposure, all bacteria in the 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, an exposure for six consecutive days will completely sterilize the fluids so exposed.

Pasteurization. It is sometimes undesirable to expose food, such as milk, to such a temperature as will destroy spores, because of the deleterious effects of such high temperatures, and yet where a partial sterilization is

necessary. Under these conditions we heat the food-stuffs for thirty minutes to such a temperature (70° C.) as will kill the bacteria in the vegetative form, but allow the spores to remain alive. Even this amount of sterilization retards greatly the rapidity of putrefaction and fermentation.

CHAPTER X.

THE DESTRUCTION OF BACTERIA BY THE CHEMICALS.

MANY chemical substances when brought in contact with bacteria unite with their cell substance. New compounds are thus formed, and the life of the bacteria and the disinfecting properties of the substances are usually destroyed. 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 as yet clear, but is apparently connected with the structure and chemical nature of their cell substance.

Chemicals are more poisonous at fairly high than at a low temperature, and act more quickly upon bacteria when they are suspended in fluids singly than when in clumps. The increased energy of disinfectants at higher temperatures indicates in itself a probability that a true chemical reaction takes place. In estimating the extent of the destructive 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*.

2. The organisms are not able to multiply, but they are not destroyed by antiseptic action.

3. The vegetative development of the organisms is destroyed, but not the spores—incomplete sterilization.

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 what is the minimum concentration of the chemical 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 are poured out into Petri dishes, where the development or lack of development of colonies and the number can be observed. 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 to each of a series of tubes holding in watery solution different percentages of the disinfectant a

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. Practically, however, the two terms are used interchangeably as meaning the destruction of all living bacteria.

few drops of the culture from which all lumps have been filtered are 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 lukewarm gelatin, from which plate cultures are made. 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 with vigorous bacteria in gelatin to which a similar trace of the disinfectant has been added.

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 only while the union exists. In some tests it is necessary to break up this union and note then whether the organism is alive or dead.

In the above determinations the absolute strength of the disinfectant required is considerably less when culture media rich in albumin are employed than when the opposite is the case. Thus creolin (Pearsons), when bouillon is used as a culture medium, stops development in the proportion of 1 to 15,000 or 1 to 5000; when ox-serum is used only in the proportion of 1 to

150 (Behring). 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.

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 ANTHRAX AND TYPHOID BACILLI IN SERUM AND BOUILLON.

Time.	1	3	5	10	20	30	45	1 hr.	1½ hrs.	2 hrs.	
A. Serum 2.5 c.c.											Solution equals 1 in 2000 bichloride.
HgCl ₂ sol. 1 : 1000 2.5 c.c.	+	+	+	+	+	+	+	—	—	—	
Anthrax thread											
B. Bouillon 2.5 c.c.											Same.
HgCl ₂ sol. 1 : 1000 2.5 c.c.	—	—	—	—	—	—	—	—	—	—	
Anthrax thread											
C. Serum 2.5 c.c.											Solution equals 2½ per cent. carbolic acid.
Carbolic sol. 5 p.ct. 2.5 c.c.	+	+	+	+	+	+	+	—	+	—	
Typhoid threads											
D. Bouillon 2.5 c.c.											Same.
Carbolic sol. 5 p.ct. 2.5 c.c.	+	+	+	+	+	+	+	—	—	—	
Typhoid threads											

— Indicates total destruction of bacteria with no growth in media.

+ Indicates lack of destruction of bacteria with growth in media.

Pieces of sterile thread (one inch) were placed in bouillon cultures of anthrax and typhoid bacilli for ten minutes, then removed to Petri dishes, and dried in the incubator for twenty-four hours. These were then placed in serum and bouillon respectively (2.5 c.c.). From each a control was taken. Then 2.5 c.c. HgCl_2 (1:1000) and carbolic solution (5 per cent.) was added to either, as shown in A, B, C, and D. From each one thread was taken at varying periods of time and planted in bouillon tubes. The threads from A and B (HgCl_2 solution) were washed in sterile water, then in a solution of ammonium sulphide (25 per cent.), then in sterile water again, then in the bouillon. The threads from the carbolic solution were washed in sterile water before planting.

Observations: The serum seems to have an inhibitory action with the bichloride solution, allowing a growth up to forty-five minutes, while with bouillon the action is much quicker, preventing a growth after an exposure of one minute or over. With the carbolic acid solution the serum seems to have made little or no difference in the results.

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 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.

A BRIEF DESCRIPTION OF SOME OF THE MORE COMMONLY USED DISINFECTANTS.

Bichloride of Mercury. This substance, when present in 1 part in 1,000,000 in nutrient gelatin or bouillon, prevents the development of parasitic bacteria. In water 1 part in 500,000 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 1000 may be required. Spores are killed in 1 to 1000 watery solution within one hour. Corrosive sublimate, as seen in the figures given above, is less effective as a germicide in alkaline fluids containing much albuminous substance than in watery solution. In such fluids, beside 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, tissue-fluids, etc., the soluble compounds of mercury are converted into oxides or hydroxides. The soluble compounds can, of course, remain in solution only when there are present sufficient quantities of certain bodies which render solution possible. Bodies of this sort are especially the alkaline chlorides and iodides, and, above all, sodium chloride and ammonium chloride. A very simple way of preventing precipitation of the mercury, then, is to add a suitable quantity of common salt to the corrosive sublimate. Those compounds of mercury which, like the cyanides, are not precipitated with alkalis, because they at once form double salts, require no addition of salt.

For ordinary use, where corrosive sublimate is em-

ployed, solutions of 1 to 500 and 1 to 1000 will suffice, when brought in contact with bacteria in that strength, to kill the vegetative forms within fifteen minutes, the stronger solution to be used when much organic matter is present.

Biniodide of Mercury. This salt is very similar in its effects to the bichloride. It is even somewhat more powerful.

Nitrate of Silver. Nitrate of silver in solution has about one-fourth the value of the bichloride of mercury as a disinfectant, but nearly the same value in inhibiting growth.

Sulphate of Copper. This salt has about 5 per cent. of the value of mercuric chloride.

Sulphate of Iron. This is a very feeble disinfectant.

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. Sodium carbonate kills spores with difficulty even in concentrated solution, but at 85° C. it kills spores in from eight to ten minutes. 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 $\text{Ca}(\text{OH})_2$ is a powerful disinfectant; the carbonate, on the other hand, is almost of no effect. 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 feces or other filth and mixed with them will completely sterilize them within one hour.

The 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 to 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.

Numerous experiments have been made with sulphur dioxide gas (SO_2), 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 not in spore 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.

Peroxide of Hydrogen (H_2O_2) 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 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 micro-organisms in a 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.

Chloride of Lime. The efficacy of chloride of lime depends on the chlorine it contains in the form of hypochlorites. A solution in water of 0.5 to 1 per cent. of chloride of lime will kill most bacteria in one to five minutes. A 5 per cent. solution usually destroys spores within one hour.

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.

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:



Formaldehyde is a gaseous compound having the chemical formula CH_2O and 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 alcohol; it exists in the solution of commerce—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.

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 ammoniacal-aldehyde, 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. Formal-

dehyde also forms combinations with certain aniline colors—viz., fuchsin and safranin—the shades of which are thereby changed or intensified. 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, and more so by its solution; 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, however, as a preservative of pathological and histological specimens.

There are no exact experiments recorded of the physiological action of formaldehyde on the human

subject when taken internally. Slater and Rideal¹ report that 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 lachrymation, 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 accidentally 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.

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 pathogenic bacteria in a few minutes. The researches of Pottevin and Trillat have shown that the germicidal

¹ *Lancet*, April 21, 1894.

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 higher 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, by an exposure of a few hours to a saturated atmosphere of formaldehyde gas.

Stahl¹ has shown that bandages and iodoform gauze can be kept well sterilized by placing in the jars containing them pieces of “formolith,” 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 $\frac{1}{10000}$ of the aqueous solution (40 per cent.), equal to $\frac{1}{25000}$ 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 minutes. In our experiments formalin has upon the vegetative

¹ Pharmaceutische Zeitung, No. 22, 1893.

forms about one-third the strength of pure carbolic acid.

Chloroform. This substance, even in pure form, does not destroy spores, but it does bacteria in vegetative form, even in 1 per cent. solution. Chloroform is used practically in sterilizing and keeping sterile blood-serum, which can be used later for culture purposes by driving off the chloroform.

Iodoform. 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 partly act destructively upon the bacteria and partly unite with the poisons already produced.

Carbolic Acid (C_6H_5OH). 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 [$C_6H_4(CH_3)OH$] is the chief ingredient of the so-called "crude carbolic acid." This is almost in-

soluble in water, and has, therefore, little 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 is a refined mixture of the three cresols (meta-, para-, and ortho-). It is soluble in water to the extent of 2.5 per cent., and is about three times the strength of carbolic acid.

Aniline Dyes. Some of these colors possess marked germicidal qualities. According to observers, methyl-violet (pyoktanin) and malachite-green destroy the typhoid bacillus in bouillon cultures in the proportion of 1 to 200 in two hours' exposure, and the pyogenic cocci in less. In 1 to 100,000 solutions they are said to retard the development of bacteria.

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

Camphor has very slight antiseptic action.

Creosote in 1 to 200 kills many bacteria in ten minutes; 1 to 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. Sandal-wood required twelve hours.

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

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

TABLE OF ANTISEPTIC VALUES.¹

Alum	1 : 222	Mercuric chloride . . .	1 : 14300
Aluminium acetate . .	1 : 6000	Mercuric iodide . . .	1 : 40000
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 : 25000
Carbolic acid	1 : 333	Quinin sulphate . . .	1 : 800
Chloral hydrate	1 : 107	Silver nitrate	1 : 12500
Cupric sulphate	1 : 2000	Sodium borate	1 : 14
Ferrous sulphate	1 : 200	Sodium chloride	1 : 6
Formaldehyde (40 per cent.)	1 : 10000	Zinc chloride	1 : 500
Hydrogen peroxide . . .	1 : 20000	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 to permanently inhibit any bacterial growth.

CHAPTER XI.

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 is 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 micro-organisms, 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 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 in it the sick person.

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.

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 methods of disinfection by chemicals described below, one ounce of common soda should be added to twelve quarts of hot soap (soft soap) and water.

2. **Strong Soda Solution.** This, which is a stronger and more effective cleansing solution and also a feeble 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. 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 "crude carbolic acid" should not be used, as it does not readily enter 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 must be kept in glass, earthen, or wooden vessels (not in metal vessels). For safety it is well to cover 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 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 one part of formalin to ten of

water. This equals in value the 5 per cent. carbolic acid solution.

9. **Creolin, Tricresol, and Lysol** are of about the same value as pure carbolic acid.

The proprietary disinfectants, which are so often widely advertised 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.

[NOTE.—The cost of the carbolic 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, generally is to be much 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.]

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, yellow fever, and cholera.

1. **Hands and Person.** Dilute the carbolic solution with an equal amount of water or use the 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 soap-suds solution for one hour. 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 when cholera is prevalent all persons should avoid eating uncooked fruit, fresh vegetables, and ice. Instead of boiling milk may be heated to 80° C. for one-half hour.

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 or bichloride of mercury solution, or milk of lime, or they should be removed on pieces of cloth, which are immediately immersed in one

of these solutions. 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 solution used to disinfect discharges should be at least twice as great as that of the discharge. 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 feces 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 fecal 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 up 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. **The 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 it can be avoided; if done, one pint of carbolic solution 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. For this purpose 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. Woodwork, floors, and plain furniture will be thoroughly washed with the soapsuds and bichloride solutions.

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 a 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 aid 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 or bichloride 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 carbolic solution should be frequently thrown in the sink after it has been flushed.

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

5. **Cellars and Rooms in Cellars** are to be frequently whitewashed, and, if necessary, the floors sprinkled with dry chloride of lime. *Areas and paved yards* should be cleaned, scrubbed, and, if necessary, washed with the bichloride solution. *Street gutters and drains* should be cleaned, and, when necessary, sprinkled with chloride of lime or washed with milk of lime.

6. **Air-shafts.** Air-shafts should be first cleaned thoroughly and then whitewashed. To prevent ten-

ants throwing garbage down air-shafts it is sometimes advisable to put wire netting outside of windows opening on shafts. Concrete or asphalt bottoms of shafts should be cleaned and washed with the bichloride solution or sprinkled with chloride of lime.

7. **Hydrant Sinks, Garbage Receptacles, and Garbage and Oyster-shell Shutes and Receptacles** should be cleaned daily and sprinkled with dry chloride of lime.

8. **Refrigerators and the Surfaces Around and Beneath Them, Dumb-waiters, etc.,** may be cleaned by scrubbing them with the hot soapsuds solution.

9. **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 solution.

10. **Urinals and the Floors Around and Underneath them** should be cleaned twice daily with the hot soapsuds solution, and in addition to this, if offensive, they may be disinfected with the carbolic solution.

11. **Stable Floors and Manure Vaults.** Stable floors should be kept clean and occasionally washed with the hot soapsuds or the hot strong soda solution. Powdered fresh chloride of lime or formalin may be used in manure vaults.

12. **Vacant Rooms** should be frequently aired.

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

14. **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.

15. Elevated and Surface 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.

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 and Sulphur Dioxide Gases in the surface disinfection of rooms and the disinfection of goods which would be injured by heat. Formaldehyde gas has so recently come into 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 micro-organisms 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 the gas in the surrounding atmosphere to kill the micro-organisms 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 micro-organisms are to be destroyed, there will be required, according to the method used, 6 to 10 ounces of formalin of full strength, or its equivalent, to 1000 cubic feet.

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 heat added.

The 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, yet 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 of walls and goods to be disinfected with water 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 12 ounces of formalin are used for each 1000 cubic feet, five hours' exposure is amply sufficient, most bacteria being killed within the first few minutes. For the destruction of micro-organisms 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, as each of these is lauded by its maker as the best; it may be of interest to give the results obtained by us from those in most common use. 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.

From 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.

In spite of present failures, it is, however, probable that in the future this method may become practicable.

From Formochloral by the Trillat System. This system consists in heating, under three atmospheres of pressure, a solution of formaldehyde gas in water mixed with 30 per cent. of calcium chloride, known as "formochloral," to a temperature of 135° C. (255° F.). It is claimed for this method of producing the gas from formochloral that the polymerization of the formaldehyde is prevented, which would otherwise take place if a solution of formaldehyde were evaporated under ordinary conditions, and that thereby the whole of the formaldehyde is obtained in the gaseous state. The addition of any neutral salt aids the process, it is said, but calcium chloride is the best. The results with this apparatus have been satisfactory, but not more so than by other methods. The apparatus is expensive and heavy.

From Formalin by the New York Sanitary Construction Company's System. This system consists in heating the ordinary commercial formalin to a temperature of about 1000° F. 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 to partially convert the methyl-alcohol contained in commercial formalin into formaldehyde gas by partial oxidation, thereby preventing the formation of methylal and increasing the amount of formaldehyde.

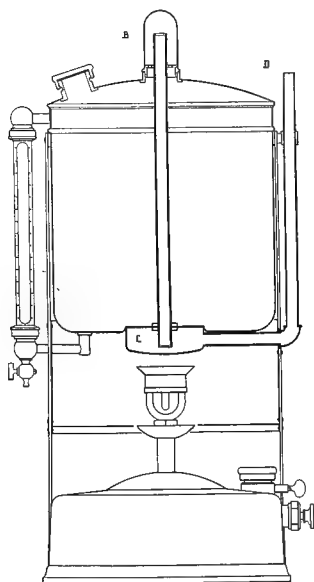
The apparatus consists of a closed receiver of copper holding about a gallon, a coil of copper pipe attached at one end to the bottom of the receiver, and, like the preceding apparatus and that made by Lentz, at the other, by means of a suitable connection (rubber tube with gutta-percha or metallic mouth-piece), with the room or apartment to be disinfected, and a heating lamp (Swedish lamp or Bunsen burner). 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 results with this apparatus have been as good as those obtained by the Trillat or Lentz systems. 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.

A great improvement in this apparatus has recently

been made by the originator of the previous apparatus, Mr. Taylor, partly through our suggestions.

In the new form (Fig. 15) 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 into the room. In this apparatus

FIG. 15.



Formaldehyde apparatus.

there is nothing to get out of order, and it operates quickly. Up to the present time this is the most practical apparatus we have met with, when the initial cost, about \$25.00, is not an objection. It is handled by H. K. Mulford Company, Philadelphia. 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.

From Trioxymethylene by Schering's System. This system consists in heating the solid polymer of formaldehyde (trioxymethylene) in a lamp specially constructed for the purpose by the Chemische Fabrik auf Actien, in Berlin. 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 gramme.

The mode of using the apparatus is very simple: The disinfecter 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 disinfecter 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 Formalin to which Glycerin has been Added. A very convenient apparatus of somewhat greater cost than that of Schering's is prepared by Charles Lentz & Sons, of Philadelphia. 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 passes over in fluid form, and is thus wasted.

With a slightly greater amount 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 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. A still simpler method is to hang sheets in a room and throw on them six to twelve ounces of formalin for each 1000 cubic feet, and leave for six hours. If the room is tightly sealed very fair superficial disinfection will take place.

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. 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 6 to 12 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 52° F. 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 30 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 110° 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 book-cases of houses and 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 be easily 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 thirty minutes.

5. Advantages of Formaldehyde Gas over Sulphur Dioxide for the 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., fifteen to thirty 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 in 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 seems to suffice 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 to more readily 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 thoroughly 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 can be 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° . 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.

The Skin of the Patient. This is washed thoroughly with soap and water, then with alcohol, and finally with 1 : 1000 bichloride. A soap poultice is now placed on for six to twelve hours, and after its removal the skin is covered with a gauze compress previously moistened with a 1 : 1000 bichloride of mercury solution. At the operation the skin is washed off with alcohol and then with the bichloride of mercury solution.

The Hands. Furbinger'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 alcohol for one minute and scrubbed with a sterile brush. They are finally soaked in a 1 : 1000 bichloride of mercury solution for three minutes.

Sterilized rubber gloves are now being used more and more in operations. The gloves can be sterilized by being left for one minute in boiling 1 per cent. soda solution, or they can be sterilized by steam.

The surgeon's gowns and caps are sterilized by steam. Mucous membranes, as 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.

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 syringes and other syringes 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 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 matter before using the carbolic acid to sterilize. Syringes made entirely of glass or of glass and asbestos can be boiled in soda solution.

THE STERILIZATION OF MILK.

Bacteria when allowed to develop in milk produce fermentation (souring) and render the milk unfit to

be used as an article of food, especially for infants. Milk as it reaches the city contains enormous numbers of germs, and these will produce fermentation, even though the milk is kept on ice. Unclean vessels hasten this process. No matter how good milk may be in the morning, when comparatively fresh, toward evening, unless it has been partly or completely sterilized, it may be dangerous to an infant, and may, especially in summer, cause fatal illness, even though it still tastes sweet.

Complete sterilization destroys all the germs in milk, and so prevents permanently fermentative changes. 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 in the warmest weather.

Milk is best sterilized by steam, for nearly all chemicals, such as boric acid, salicylic acid, and formalin, make the milk less digestible, and, as a rule, unfit for food. It may be sterilized at a high or low temperature—that is, at the boiling temperature (212° F.)—or at a lower degree of heat, obtained by modifying the steaming process.

It has been found that milk sterilized at a high temperature (212° F.) is not desirable for prolonged use, as the high temperature causes certain changes in the milk, which make it less suitable as a food for infants. 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 70° C. for fifteen minutes or 80° C. for twelve minutes will accomplish this. 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 in the shops for this purpose).

(c) Rubber corks for the bottles and a bristle brush for cleaning them.

Directions (Koplik). Place the milk, pure or diluted (as the doctor 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 of sterilization 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 188° F. (87° C.).

Milk should be sterilized when it is as fresh as possible, and only sufficient milk for twenty-four hours should be sterilized at one time. If, after nursing, the infant leaves some milk in the bottle, this should be thrown away.

Care of the Bottles is Important. 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 should be boiled after using 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.

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 75° C. (167° F.), and holds it nearly at that point for some time.² After twenty minutes the milk is removed, placed in cold water, and quickly cooled. The milk is kept in the ice-chest until used.

¹ Agent for Pasteurizer, James Dougherty, 411 W. 59th St.

² A temperature of 75° C. is advised in Pasteurizing milk, instead of 65° C., which would ordinarily suffice to kill all bacteria free of spores, because of the fact pointed out by Theobald Smith, that the bacteria embedded in the pellicle which forms on the surface are more resistant than those surrounded by fluid.

CHAPTER XII.

THE PREPARATION, STAINING, AND MICROSCOPICAL EXAMINATION OF BACTERIA.

As the purpose of this book is to give, outside of special methods devised for purposes of diagnosis and the development of curative serums, only such descriptions of technique as are necessary to students in their laboratory courses, or to physicians in the very simple examinations which they will be able to carry on in their private offices, readers are referred to works such as Sternberg's or Abbott's for fuller descriptions of the apparatus and technique used in bacteriological research.

Since bacteria are present to a greater or less extent in the air, earth, and water around us, on our bodies, clothes, and all surrounding objects, it follows that when we begin to examine substances for bacteria the first requisite is that all the materials we use must be free and kept free from bacteria, both living and dead, otherwise we cannot tell whether those we detect are in the substances examined or only in the materials we have used in the investigation.

Additional care has to be taken when we study infection in the living body, for in the skin and mucous membranes there are not only abundant bacteria but varieties similar to those which produce disease, so that if we do not use the greatest precautions we will contaminate our material with these bacteria and get utterly

misleading results. After death we have an added difficulty, in that even the blood and body tissues become invaded by bacteria from the intestines and elsewhere, so that bacteria actually present in the diseased tissues may have had no connection with the disease under investigation. Whenever bacteria are found, therefore, the methods carried out in the investigation should be most carefully examined, to see if some error in technique has not been committed. The aim of the bacteriological examination of any material is to determine whether bacteria are present or not, and, if present, to ascertain their number and distribution, and, if possible, their species. This is accomplished chiefly by means of two methods—viz., the direct examination with the microscope of cover glass preparations and the results of cultures made from the material. Sometimes animal inoculations are also employed.

The direct microscopical examination of suspected substances for bacteria can be made either with or without staining. Unstained, the bacteria are examined, to note their motility, their form, and their general arrangement: but for more exact study, they can be so much better observed when stained that this step is always advisable.

A cover-glass preparation is made as follows: A very small amount of the blood, pus, discharges from mucous membranes, culture fluids, or other material to be examined is removed by means of a sterile swab or platinum loop and smeared undiluted in a thin film over a clean, thin cover-glass.¹ From cultures on solid

¹ To render new cover-slips clean and free from grease, place them in strong nitric acid for a few hours, then rinse them off in water, then in alcohol, then in ether. Place them finally for keeping in alcohol, to which a little ammonia has been added. When used wipe with soft clean handkerchief. If old cover-slips are used boil first in soda solution.

media, however, on account of the abundance of bacteria in the material, a little of the growth is diluted by adding it to a tiny drop of clean water which has been previously placed on the cover-glass. The amount of dilution is learned after a few trials. It is best to add to the drop just enough to make a perceptible cloudiness. The mixture is then smeared over the cover-glass. From whatever source derived, the film is allowed to dry at the usual air temperature, and then, in order to fix the film with its contained bacteria to the glass, the latter 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 by placing it in absolute alcohol 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 one to five minutes, or by one of the more complicated special stains described later, such as that of Gram or that used for the tubercle bacillus, where the ability of the bacteria to retain their stain when placed in decolorizing solutions is tested. 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. The cover-glass, 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 it may be held in the Cornet forceps. The fluid in both the dish and on the cover-glass should be carefully warmed, so as to steam without actually boiling. The cover-glass

should be kept completely covered with fluid. The bacteria having now been stained, the cover-glass is grasped in the forceps and thoroughly washed in clean water and then dried, first between layers of filter-paper and then in the air. A drop of balsam or water is now placed on a glass slide and the cover-glass placed upon it with the bacterial side down. The preparation is now ready for microscopical examination.

The Preparation of Sections. Occasionally it is of value to examine the bacteria as they are in the tissues themselves. These should be obtained as soon as possible after death, so as to prevent any post-mortem changes or increase of the bacteria in them. From the properly selected spots small portions, not larger than one cubic centimetre, are removed and placed in absolute alcohol to harden. These portions, when of nearly the consistency of fresh, solid rubber, are removed, and, if the nature of the tissues will allow, fastened to corks or pieces of hard rubber by mucilage. After drying, the specimens are replaced in alcohol for twenty-four hours and then cut into thin sections with the microtome. Sometimes the tissues do not become sufficiently dense by this simple method; they must then undergo the process of embedding in celloidin or paraffin.

The Ordinary Staining Solutions. Simple staining is used for the demonstration of bacteria in general, and is also useful for gaining an idea of the other elements in the preparation. The solutions commonly employed in staining bacteria are the watery solutions of basic aniline dyes — fuchsin, gentian-violet, and methylene-blue. These solutions may either be prepared by dissolving the dyes directly in water, or, more usually, by having stock saturated alcoholic solutions of them, from which

we can take from time to time the amount necessary to make up the watery solutions for use. The stock saturated alcoholic solutions are made by pouring into a bottle enough of the dye in substance to fill them to about one-quarter of their capacity. The bottle should then be filled with alcohol, tightly corked, well shaken, and allowed to stand for 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 will then be labelled "saturated alcoholic solution," of whatever dye has been employed. The alcoholic solutions are not themselves 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. Care must be taken that the color does not become too dense. Small wooden cases come prepared for holding about one-half dozen bottles of the staining solutions. This number will answer for all routine purposes of the student or physician.

For certain bacteria, which stain only imperfectly with these solutions, it is necessary to employ some agent that will increase the penetrating action of the dyes. We have learned that the addition to a solution of a small quantity of alkaline substance, or by dissolving the staining materials in strong watery solutions of either aniline oil or carbolic acid, instead of simple water, will accomplish this. Of the solutions thus prepared there are three in common use: Loeffler's

alkaline methylene-blue solution, the Koch-Ehrlich aniline water solution, of either fuchsin, gentian-violet, or methylene-blue, and Ziehl's solution of fuchsin in carbolic acid. These solutions are as follows :

Loeffler's Alkaline Methylene-blue Solution. This consists of concentrated alcoholic solution of methylene-blue, 30 c.c.; caustic potash in one ten thousandth solution, 100 c.c.

Koch-Ehrlich Aniline Water Solution of Fuchsin or Gentian-violet is prepared as follows: To about 100 c.c. of distilled water, aniline oil is added, drop by drop, until it has an opaque appearance, the solution being thoroughly shaken after the addition of each drop. It is then filtered into a beaker through moistened filter-paper until the filtrate is perfectly clear. To 100 c.c. of the filtrate add 10 c.c. of absolute alcohol and 11 c.c. of the concentrated alcoholic solution of either fuchsin, methylene-blue, or gentian-violet.

Ziehl's Carbolic Fuchsin Solution. Distilled water, 100 c.c.; carbolic acid (crystalline), 5 grains; absolute alcohol, 10 c.c.; fuchsin, 1 grain; 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 last two methods, combined with heating, are used to stain the bacteria intensely, so that the more resistant of them may retain their color when exposed to decolorizing agents. When so treated certain of the bacteria will retain their color, even when exposed to very strong decolorizers. The bacilli of tuberculosis and of leprosy are examples. They are both difficult to stain, but when once stained are equally resistant to

give up their stain. The details of staining tubercle bacilli will be found under Tuberculosis.

Another differential method of staining which is very commonly employed is that known as Gram's method. In this method the objects to be stained are covered with the aniline gentian-violet solution. After remaining in this for a few minutes they are immersed in an iodine solution, composed of iodine, 1 grain; potassium iodide, 2 grains; distilled water, 300 c.c. In this they remain for from one to two minutes. They are then transferred to alcohol and thoroughly rinsed. If the cover-glass as a whole still shows a violet color, it is again treated with the iodine solu-
~~tion and alcohol and this is continued until~~

ERRATA.

Thirteenth and fourteenth lines from bottom, *for* grains *read* grammes.

in the iodine solution for a suitable time.

Another method of demonstrating the capsule is the glacial acetic acid method, as described by Welch:

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 2 per cent. solution of sodium chloride and mount in the same.

Staining the Spores. 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

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Another method of demonstrating the capsule is the glacial acetic acid method, as described by Welch:

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 2 per cent. solution of sodium chloride and mount in the same.

Staining the Spores. 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 stains have been devised for causing the color to penetrate through the resistant spore. Thus in Abbott's method the cover-slip after having been prepared in the usual way is covered with a dye 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, until all visible color has disappeared, then it is washed off and dipped for ten seconds in a solution containing 10 parts saturated alcoholic solution of eosin and 90 of water. The bacilli will then be rose-colored and the spores blue. Sometimes, however, 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. He macerates the spores in a solution of chromic acid before staining them. The prepared cover-slip is held for two minutes in chloroform, then washed off in water, then placed from one-half to three minutes in a 5 per cent. solution of chromic acid, again washed off in water, and now restained by adding to it 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 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 they 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 to Loeffler. The special stains devised by him, and also the one devised by Van Ermengem, are those usually employed.

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 of an eighteen-hours' old culture of the motile organism to be examined. 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 alum solution add 1 part of a solution of liquor ferri sesquichloride, of the strength of 1:20 of distilled water. To 10 c.c. of this mixture add 1 c.c. of a concentrated watery solution of fuchsin. This mordant should be allowed to stand for several days 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 slightly warmed and then washed off. After drying the smear is faintly stained with the carbol fuchsin solution and then washed off, dried, and mounted.

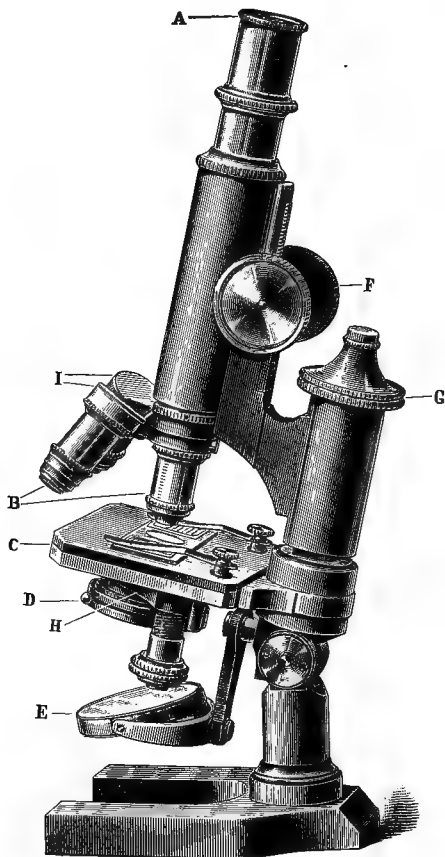
Frequently the flagella appear well stained, but often the process has to be repeated a number of times before success is arrived at.

The Preservation of Specimens. Dry unstained or stained preparations of bacteria keep indefinitely if mounted in Canada balsam, cedar oil, or dammar lac; they tend to gradually fade, but may be preserved for many months or years.

The Microscopical Examination of Bacteria.

The Different Parts of the Microscope. A complete instrument usually has four oculars, or eye-pieces (A), which are numbered from 1 to 4, according to the

FIG. 16.



Microscope.

amount of magnification which they yield. Numbers 2 and 4 are most useful for bacteriological work. The objective (B)—the lens at the distal end of the barrel—serves to give the main magnification of the object. For stained bacteria the 1/12 achromatic oil immersion lens is regularly employed; except for photographic purposes the apochromatic lenses are not needed. Even here they are not indispensable. A 1/10 or 1/16 may at times be useful but hardly necessary; a No. 4 ocular and a 1/12 lens give a magnification of about 1000 diameters. (Fig. 17.) For unstained bacteria we employ

FIG. 17.

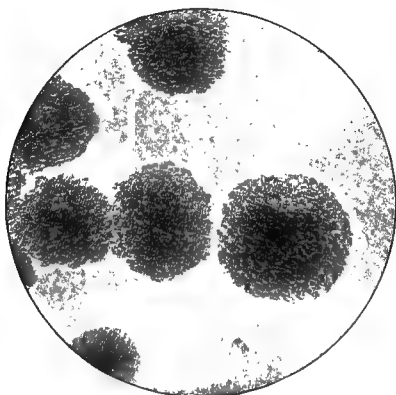
Anthrax bacilli and blood-cells. $\times 1000$ diameters.

either the 1/12 immersion or 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. 18.)

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 iris diaphragm (D), which is now regularly used in bacteriological work, opens and closes like 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 placed beneath the stage serves to direct the light to the object to be examined. It has two surfaces—one concave and one convex. The coarse ad-

FIG. 18.

Colonies of diphtheria bacilli. $\times 200$ diameters.

justment (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. 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 high-power lenses are used. For the microscopical study of bacteria it is essential that we magnify the bacteria as much as possible and still have their definition clear and

sharp. It is essential, therefore, that the microscope be provided with an oil immersion system and a substage condensing apparatus. In using the oil immersion lens a drop of oil 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 light through deflection, as is the case in the dry system.

The 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 focussed upon the object, thus furnishing the greatest amount of luminosity. Between the condenser and the reflector is placed an adjustable diaphragm, the aperture of which can be regulated, as circumstances require, to permit of either a very small or a very large amount of light passing to the object.

The Examination of Bacteria in the Hanging Drop. 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,

FIG. 19.



Hollow slide with cover-glass.

whether they move and produce spores, and whether or not they clump or disintegrate with specific serums. For this special slides and methods are desirable. The usual form is one in which there is ground out on one surface a hollow having a diameter of about half an inch. (Fig. 19.) 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 vaseline or other inert substance. This has for its purpose both the sticking of the cover-glass to the slide 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 platinum loopful upon the centre of the cover-glass and then invert it by means of a slender pair of forceps over the hollow in the slide, being very careful to have the bacteria over the very centre of the space. 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 dif-

ferent materials under examination. It is necessary to remember that they are seen with difficulty, and that we are very apt, unless extremely careful in focussing, 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 raise the lens, for it has gone beyond the point of focus and is touching the cover-glass.

CHAPTER XIII.

BACTERIOLOGICAL TECHNIQUE—*Continued.*

THE CULTIVATION OF BACTERIA.

IN order to determine the number of living bacteria in any substance and their nature we have to cultivate and isolate them.

The Most Common of the Nutrient Media Used for the Growth of Bacteria.

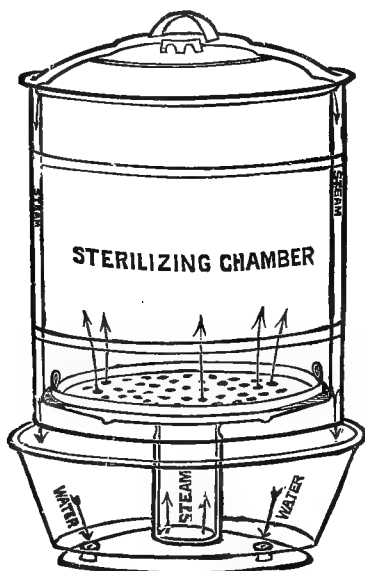
All of these must have, as noted earlier, food containing the necessary carbon, nitrogen, and mineral substances in a form easily assimilated and in the proper concentration. The pathogenic bacteria nearly all require for good growth peptone, albumins, and sugar. For each kind the proper food must be found through experimentation, as slight alterations may make a great difference.

Physicians will find it, as a rule, convenient to purchase their media already prepared from some of the reliable firms that deal in bacteriological products. Special media, such as those employed for isolation and identification of the typhoid bacillus and gonococcus, will be found described along with those bacteria. For those who may wish to make their own, we will describe here those in common use:

Nutrient Bouillon or Broth. One part of finely chopped fresh, lean meat is macerated in two parts of water and

put in an ice-chest for from eighteen to twenty-four hours. The infusion is strained, when cold, through a fine cheese-cloth, and to the clear filtrate 1 per cent. of peptone and 0.5 per cent. of sodium chloride are added. The medium is then warmed for some minutes until the peptone is dissolved, and then exposed to live steam either without pressure in the Arnold steam sterilizer (Fig. 20) for thirty minutes, or in the autoclave (Fig.

FIG. 20.

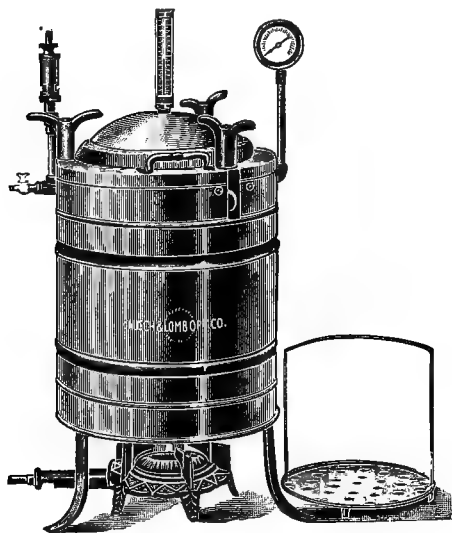


Arnold steam sterilizer.

21) at one atmosphere of pressure for fifteen minutes, or boiled over a free flame for ten minutes. While still hot it is filtered through filter-paper or through absorbent cotton, and the reaction is tested and sufficient hydrochloric acid or sodium hydroxide added

to give it the desired reaction, which is for most bacteria slightly alkaline to litmus. If the fluid is clear it is put into flasks and tubes and sterilized; if not clear, the white of one or two eggs is added to the fluid after cooling it down to about 55° C. After

FIG. 21.



Autoclave for sterilization with live steam under pressure.

thoroughly mixing the eggs the bouillon is boiled briskly for a few minutes and then again filtered and distributed in flasks and tubes and put in the Arnold sterilizer for one hour on each of two consecutive days, or in the autoclave for twenty minutes for sterilization. Instead of meat 2 to 4 grammes of Liebig's or some other meat extract are added to each litre of water. For most purposes the extract is as good as the fresh meat, but for toxin production it is inferior.

Fermentation broth is made usually by adding 1 per cent. of glucose to the above. For accurate work the meat sugars are first extracted by allowing the colon bacillus to grow in the broth over night. The bouillon is then sterilized and the peptone and salt added, and the process already given gone through with.

Fermentation bouillon is usually placed in a tube of special construction, known as a fermentation tube (see Fig. 14, p. 82). This is essentially a tube 1.5 cm. in diameter, bent at an acute angle, closed at one end, and provided with a bulb at the other end, which latter should be large enough to receive all the fluid in the closed branch should gas in any considerable quantity collect there. The tube also serves a most important end in giving information as to the aërobic and anaërobic growth of the species under consideration, for the connecting tube being constricted serves to prevent, to a great degree, the entrance of oxygen of the air into the closed branch, and the free oxygen in the medium is driven out by the heat during sterilization; from which it may be seen that growth in the bulb is aërobic and growth in the closed branch is anaërobic. For the study of fermentation alone small tubes may be inverted into larger ones or tubes may be bent on themselves.

Nutrient Gelatin. To the 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. 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.

Nutrient Agar. This is prepared by adding to stock

bouillon 1 or 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. 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. 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.

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 a steam sterilizer for fifteen minutes and then 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.

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, 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. For more careful work the potatoes are first cut into proper sizes for tubes or

dishes, and then soaked for from twelve to eighteen hours in running water; this removes excessive acidity; they are then placed in test-tubes and sterilized by steam on two consecutive days.

Blood-serum and Ascitic Fluid with and without the Addition of Bouillon. Blood-serum is used in the fluid state, semi-solid, and firmly coagulated. It is used alone, with 66 per cent. of bouillon and with 25 per cent. of bouillon plus 1 per cent. of glucose. Ascitic, pleuritic, and hydrocele fluids are also used alone, with bouillon, or with nutrient agar.

The Correction of the Reaction in Media.

Formerly it was customary to use litmus-paper as the indicator in neutralizing media, adding soda solution until the mixture turned the red litmus slightly blue, and the blue litmus just a tinge less blue. This is still the best method for those who are only going to cultivate the common pathogenic bacteria for diagnostic purposes or for the development of toxin. Most parasitic bacteria which grow at all on artificial culture media develop best in them when they have a slightly alkaline reaction to litmus. If a greater alkalinity is desired a certain number of c.c. of normal soda solution can be added for each litre; 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. For these purposes phenolphthalein has been generally selected. A little experience will show that different indicators not only differ in delicacy, but that they react differently to different substances.

A litre of bouillon becomes on the addition of 1 per cent. of peptone more alkaline to litmus, but decidedly more acid to phenolphthalein. We have, therefore, especially with the latter substance, to find by growing the bacteria just what reaction we want, and then test the fluid with phenolphthalein as the indicator. With exactly similar materials we can exactly reproduce at any time in the future the same reaction, but with different materials this would be impossible. A bouillon which contains 1 per cent. of peptone and reacts neutral to litmus is about 15 points acid to phenolphthalein—that is, 15 c.c. of normal soda solution must be added per litre to make the bouillon neutral.

When phenolphthalein is used we must have accurately standardized solutions of caustic soda and hydrochloric acid. The test is carried out as follows: To 10 c.c. of the hot nutrient bouillon add one drop of a 1 : 300 solution in alcohol of phenolphthalein; into this is dropped slowly a 4 per cent. solution of caustic soda until a faint rose-tint appears. This indicates the beginning of an alkaline reaction. To make a litre neutral we would add 100 times as much of the decinormal solution of caustic soda as was required to make 10 c.c. neutral. As a rule, we use 1 per cent. peptone bouillon of such an acidity that 15 c.c. of normal soda solution must be added to each litre to make it neutral.

The Sterilization of Different Media.

Flasks and tubes of nutrient broth and agar are easily sterilized by placing them in an Arnold steam sterilizer (Fig. 20) for from fifteen minutes to one hour, according to the bulk of the fluid, upon two or three consecutive days. They can also be even more cer-

tainly sterilized by putting them in an autoclave (Fig. 21) at 110° C. for from fifteen to thirty minutes on two consecutive days.

Gelatin is sterilized in the same manner except, as already stated, the shorter times are used. Prolonged heating destroys the congealing properties of the gelatin.

Blood-serum may be sterilized by fractional sterilization and remain fluid, or may be rendered solid by the degree of heat used in sterilizing.

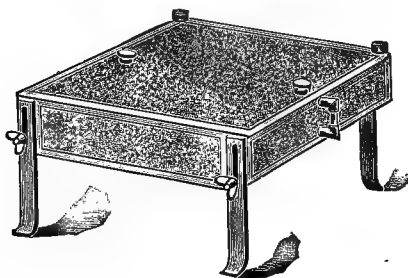
For the sterilization of fluid serum it is requisite that it be exposed to a temperature of from 62° to 66° C. for one hour on each of six consecutive days. The best apparatus for obtaining and maintaining this temperature (about 65° C.) is a small and well-regulated incubator or chamber surrounded by 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 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 temperatures to run the temperature up slowly and not to heat above 90° 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 temperatures mentioned in an incubator, water-oven, or even in an Arnold steam 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. 22) is, however, the most convenient apparatus.

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 filled into

FIG. 22.



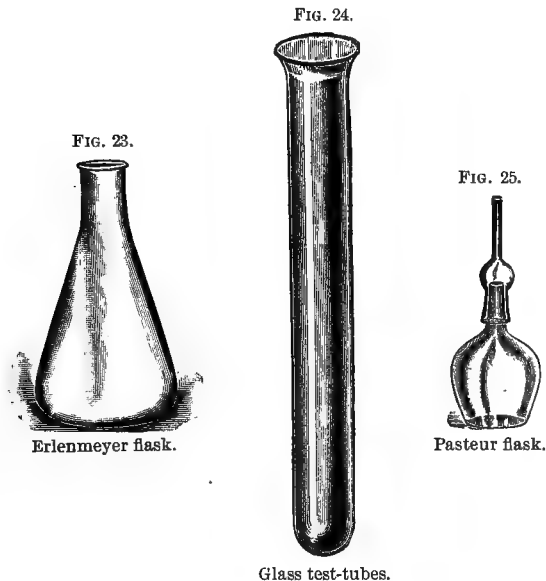
Blood-serum coagulator.

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 Pasteur or Berkefeld filter, under pressure. When so treated the fluid is very clear and light-colored.

Flasks, Dishes, Tubes, etc., Used for the Preservation of Media and for other Bacteriological Purposes. The nutri-

ent media are stored in large quantities in round or flat-bottomed Erlenmeyer flasks (Fig. 23). From these, as needed, glass tubes (Fig. 24) are filled. Glass dishes with covers (Petri dishes, Fig. 27) and flat flasks are used for growing bacteria in or upon thin layers of media. When small amounts of media are taken frequently from flasks, Pasteur's flasks (Fig. 25) 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.

The Methods of Obtaining and Studying Pure Cultures of Single Species of Bacteria.

In order to study bacteria, both in culture media and in the living body, we must separate those developed from one organism from all others and study them by themselves in pure cultures. In order to do this 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. We also carefully try to prevent any bacteria entering from the air or elsewhere.

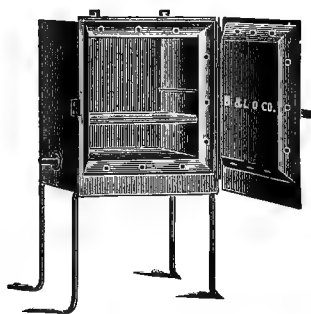
The Cleansing and Sterilization of Apparatus.

In bacteriological 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 desired to study.

The platinum wires and loops 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. 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 2 per cent. solution of nitric 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 and then thoroughly rinsed off with water until perfectly clean. If neces-

FIG. 26.



Dry heat sterilizer.

sary, 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 (Fig. 26).

The sterile tubes and flasks are filled with the media, when small quantities are used, by means of a 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 full of media, are put in the steam sterilizer for one half hour on three consecutive days, or in the autoclave for twenty minutes for 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.

Technique of Making Plate Cultures.

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 placed on solid media they develop about the spot where they were inoculated. 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. 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 it 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 at a high temperature and begins to thicken at about 36° C. It is not liquefied by bacterial ferments. Nutrient 10 per cent. gelatin melts at the low temperature of about 23° 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. To prevent this, when a number of tubes are to be inoculated they are placed while still hot in a basin of water which has been heated to about 45° C. When the temperature of the agar in one of the tubes,

FIG. 27.

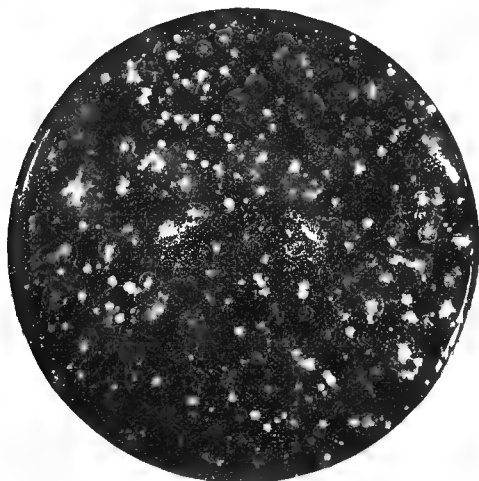


Petri dish.

as tested by a thermometer, has fallen to 40° the water, milk, feces, bacterial culture, or other substances to be tested are added to the other tubes in whatever quantity is thought to be proper. After inoculation the contents of the tubes are thoroughly shaken and poured out quickly into round, flat-bottomed glass dishes (Fig. 27), the covers of which are removed for the required time only. 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 (Fig. 28) thus indicate to us roughly the number of living bacteria in the quantity of fluid added to the liquid agar. Nutrient gelatin is used exactly as agar, except that, as it does not congeal until cooled below 22°C ., we have no fear of

FIG. 28.

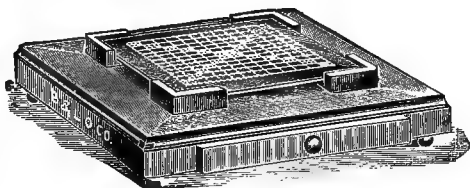


Photograph of a large number of colonies developing in a layer of gelatin contained in a Petri dish. Some colonies are only pin point in size; some as large as a pencil. The colonies here appear in their actual size.

its cooling too rapidly. In order not only to count the number of colonies which develop, but also to obtain a characteristic growth, it is desirable not to have them too near together. As it is impossible to determine accurately the number in any suspected fluid, it is usual to make a set of four different plates, to each of which a different amount of material is added, so that

some one of the four will have the required number of colonies. In the 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 colonies, the second would have 6000 (Fig. 28), the third 600, and the fourth 60. If, on the other hand, the first contained but 60, 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. 29), ruled in larger and

FIG. 29.



Wolffhügel's apparatus for counting colonies.

smaller squares. With a hand lens the colonies in a certain number of squares are counted and then the number for the whole contents estimated.

When the material to be tested is crowded with bacteria it is often best to make an emulsion of a portion of it, and use this rather than the original substance for making the cultures.

Measured quantities of the diluted material can be transferred most accurately through a sterilized long glass pipette graduated in one-hundredth cubic centimetres, or, more roughly, by a platinum loop of known size.

The nutrient agar-agar is frequently used in a different manner. A small quantity is poured into the Petri dish and allowed to harden. The substance to be tested bacteriologically, or a dilution of it, is then streaked by means of a platinum loop lightly over its surface. While in the former method 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. The method of using glass plates upon a cooling stage has now been practically given up for the more convenient one of Petri dishes. In warm weather the dishes should be cooled before using, so as to harden quickly the agar or gelatin that is poured into them.

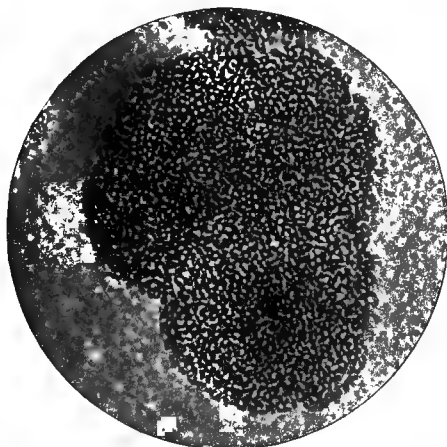
An old method, which is still sometimes used to find the number of living bacteria, is, instead of pouring out the media which has been inoculated, to congeal it on the sides of the test-tube. This is best done by laying the tube flat on its side on a cake of ice and rotating it. Tubes come especially 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.

The Study of Colonies in Plate Cultures in Nutrient Agar.

The plates should be removed after twelve to twenty-four hours' growth at blood temperature and after one to three days at 70°. 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 maximum 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 ones. As a rule, the younger colonies are more characteristic, except where the development of pigment is sought.

FIG. 30.



Two surface colonies of diphtheria bacilli upon agar. $\times 500$ diameters.

The colonies are first examined with the eye (Fig. 28), then with a low magnification, and then again at from 400 to 500 diameters (Fig. 30). We note everything we can about them, such as their size, border, density, color, and granular appearance. At the higher magnification we begin to detect the individual bacteria. After studying the colonies we remove a few of the bacteria from one or more of them by touching them with

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

The Study of Plate Cultures in Gelatin Media.

The gelatin media have one marked characteristic to be noted which never occurs upon agar—namely, some of the colonies will be lying in or surrounded by slightly opaque fluid, due to the liquefaction of the gelatin. (See paler and larger colonies in Fig. 28.) In using nutrient gelatin one must always remember not to allow it to stay where the temperature is over 20 C., for if that happens the media will melt, nor must the liquefying colonies be allowed to grow for too long a time, or the entire media will become fluid.

FIG. 31.



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

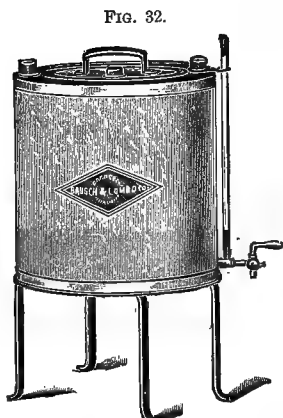
Pure Cultures. If we transfer without contamination bacteria from a colony formed from a single organism

to new media, and these grow, we have what we call 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. (Fig. 31.)

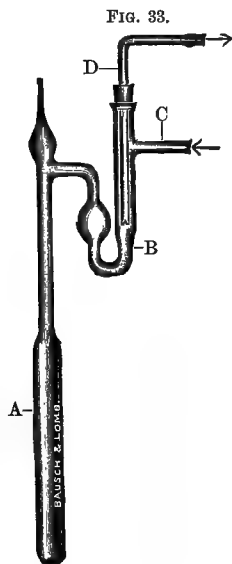
In transferring bacteria from one tube to another we slant the tubes so that no dust may fall within and contaminate with other bacteria the special variety we wish to transplant. The greatest care must be taken that the sterilized platinum needle used to transfer the bacteria is not infected by touching any non-sterile matter. 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 possibility of contamination must always be kept in mind. When it occurs upon solid media we, as a rule, easily detect it, for we notice the growth at some point 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.

Incubators. In order to have a constant and proper temperature for the growth of bacteria, forms of apparatus called incubators have been devised. These (Fig. 32) consist in their simplest form of an inner air chamber surrounded by a double copper wall containing water. The apparatus externally is lined with asbestos, to prevent radiation. It is supplied with doors and with openings for thermometers and a thermo-regulator. The thermo-regulators are of various kinds; those in most use depend upon the expansion or contraction of the fluid in a bulb (A, Fig. 33), 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 (Fig. 33).



Small incubator.



Thermo-regulator.

Other forms 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.

The temperature 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 thermo-regulator. Incubators are also both warmed and regulated by electricity.

In emergencies a culture may be developed at the blood temperature by placing it in water contained in a small vessel, which itself is contained in a larger vessel, also filled. By adding a little hot water from time to time the temperature can readily be kept between 34° and 38° C., which is sufficiently uniform for bacteria such as the diphtheria bacilli to grow.

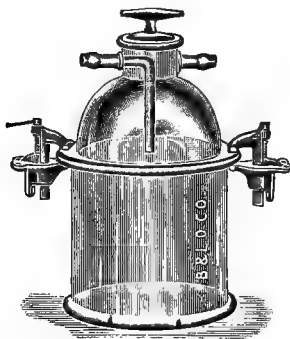
As a temporary expedient during the night, when haste is necessary, it is possible, when the culture medium is solid and within a strong glass tube or metal case, to make use of the body heat by putting it under the clothing next to the body and sleeping upon it. Naturally, this should only be done when other means fail. Several times, when in the country, this method has enabled the writer to obtain a growth of diphtheria bacilli over night, and thus get important information, when otherwise it would have been impossible.

Culture Methods for Anaërobic Bacteria.

Anaërobic bacteria will scarcely be cultivated except in bacteriological laboratories, where the technique is already understood, so that the methods employed in their culture will only be touched on here. A simple device is that of Koch, who placed a thin strip of sterile mica upon the still fluid agar or gelatin in the Petri dish, which had already been inoculated. 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 semi-solid. 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. In the more complicated methods the plates or tubes are placed in jars (Fig. 34), in which the oxygen is dis-

FIG. 34



Jar for anaërobic cultures.

placed by a stream of hydrogen developed by the Kipp apparatus through the action of pure granulated zinc and a pure 25 per cent. solution of sulphuric acid. When all the oxygen has been displaced the jars are sealed by rotating the stopper. 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 gramme of pyrogallic acid and 10 c.c. of 6 per cent. solution of potassium hydroxide are added and the jars immediately sealed. 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. 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.

CHAPTER XIV.

THE USE OF ANIMALS FOR DIAGNOSTIC AND TEST PURPOSES.

SUITABLE animals are necessarily employed for many bacteriological purposes. Thus they may be used as a soil for bacterial growth, when, as in the case of tubercle bacilli, the bacteria will not develop in the dead culture media. For this reason material suspected to contain tubercle bacilli is injected into rabbits or guinea-pigs, with the knowledge that, if present, although in too small numbers to be detected by microscopical or culture methods, they will develop their lesions in the animal's bodies, and thus reveal themselves. The same may be true of glanders and anthrax bacilli and of other bacteria. Again, animals are used to test the virulence 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 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 without and under the influence of specific serums upon them. In this way the typhoid and colon bacilli may be separated, or the pneumococcus and streptococcus. Still, a different use of animals is to measure the protective effect of antitoxic and bactericidal serums; thus, 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. The use of animals to develop through bacterial injections protective serums will be dealt with under the special bacteria by whose products they are produced.

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 hypodermatic 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 hypodermatic 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 rabbit 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 their edges. 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.

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 hypodermatic needle is usually employed, less often a glass tube drawn out to a fine point. The needle or the pointed glass tube are gently pushed through the abdominal wall, moved about to insure its freedom from the intestines, 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 injected substances into the trachea and bronchi.

8. Through the intestinal tract by swallowing.

In these injections guinea-pigs are held, as a rule, by an assistant grasping in one hand the forelegs and in the other the hindlegs.

Rabbits can be held in the same manner, or better placed in some holder.

Mice, which are usually inoculated subcutaneously 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.

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. 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.

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 cold place. 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 means of a 5 per cent. solution of carbolic acid. All instruments are sterilized by boiling in 3 per cent. soda solution for five minutes. Changes of knives 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.

When it is necessary to transport tissues some 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 vessel which contains them with ice.

Animals rarely show the same gross lesions as man when both suffer from the same infection. The cell changes are similar, and, also, so far as we can test them, the curative or immunizing effects of protective serums.

CHAPTER XV.

THE PROCURING OF MATERIAL FOR BACTERIOLOGICAL EXAMINATION FROM THOSE SUFFERING FROM DISEASE.

A LONG experience has taught me that physicians very frequently take a large 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 bacteriological 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 inoculation upon or in culture media or animals the more exact the information which will be obtained from its examination. 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, culture media should be inoculated in the neighborhood of the patient or dead body. For that purpose a bacteriologist should take the most suit-

able 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, and firmly solidified slanted blood-serum. 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 bacteriological technique. 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 or no organisms present in the blood until shortly before death. It will, therefore, be 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 3 to 5 c.c. of blood by means of a sterile hypodermatic needle, or a suitable glass tube armed with a hypodermatic needle, from the vein of the arm, after proper cleansing of the skin and a tiny incision. Into each of five different tubes containing bouillon we add one-fifth of the quantity of blood withdrawn. 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 streak several nutrient agar plates with blood, so as to indicate roughly the number of organisms present, if they happen to be in abundance.

From wounds, abscesses, cellulitis, etc., the substance for bacteriological examination can, as a rule, best be obtained by means of small rods armed with a little absorbent cotton. A number of these can be carried in a test-tube. Both rods and tubes must be sterile. 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, here 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. After having made the cultures, 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.

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 to clearly 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 bacteriological 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 feces, 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 feces in the course of even twelve hours, while at other times they may remain present for weeks. These differences depend on the associated organisms present, the chemical constitution of the feces or urine, and the conditions under which the material is obtained.

CHAPTER XVI.

BACTERIOLOGICAL EXAMINATION OF WATER AND AIR—THE CONTAMINATION AND PURIFICATION OF DRINKING WATERS.

Bacteriological Examination of Water. The bacteriological examination of water is undertaken with two purposes: First, to discover the number of living bacteria present in the water, and, second, the varieties that may be present. In order to roughly determine the number of living bacteria in water, we thoroughly mingle certain definitely measured quantities of water with suitable quantities of melted but sufficiently cooled nutrient agar or gelatin, the mixtures being immediately poured into Petri dishes, or retained in Esmarch tubes and allowed to quickly harden. The bacteria in the water are thus scattered throughout the solidified media. If nutrient gelatin is employed care must be taken to keep it cool during transportation. The agar is usually allowed to remain at the body-temperature, while the gelatin is necessarily kept at the usual room-temperature (about 70° F.). If nutrient agar alone is used two sets of plates are made, one being kept at body-heat the other at the usual room-temperature. After a suitable length of time has been allowed for the development of the colonies we examine the plate cultures, and by counting the number of colonies developed by means of a low power lens and Wolffhügel's apparatus (Fig. 29) or some equivalent, we are enabled to find approximately the number of living bacteria

which were present in the water at the moment the water was examined. Any bacteria, however, which, though living in the water, were unable to grow in the media or at the temperatures employed would, naturally, not reveal themselves by the growth of colonies, nor would bacteria clinging together in bunches count as more than a single member. As to the value of learning the number of bacteria in water, we must admit that a single determination of the number of living bacteria in any sample is now known to be of little avail unless the conditions under which the water exists are well known or the number of bacteria is enormous. Thus, for instance, the water in an Adirondack lake might contain in a cubic centimetre far more bacteria than that of a well which was slightly contaminated with typhoid bacilli from human sources. If, however, we knew the usual condition of the well water and the usual number of bacteria present in it, any sudden increase would, of course, give us a strong suspicion, but nothing more than a suspicion, of dangerous contamination. In the same way in a stream into which a sewer empties, if we find a great many more bacteria in the stream some distance below the point of entrance of the sewer than there were above, we would have every reason to believe that the increase of bacteria found in the stream below was due to the bacteria added to the stream by the sewer; if we drank that water we would know from the examination we were drinking not only a portion of the sewage but of the bacteria contained therein. It is true, that with our present knowledge, derived from previous bacteriological studies, we would be almost as certain of these facts before as after the bacteriological examination. The determina-

tion, therefore, of the number of bacteria should only be considered of value, except in the extreme instances where enormous numbers are found, when we know fairly well the conditions, chemical and physical, concerning the supply. The examination of water, to determine whether or not any forms of parasitic bacteria or other micro-organisms are present, would be more often of practical value than it is if the difficulties were not so great. As a matter of fact, water examinations for this purpose are usually negative. The varieties of bacteria most sought, except in the presence of a cholera epidemic, are the typhoid and colon bacilli. 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. In our experience this and other methods have not enabled us to detect the typhoid bacillus where we have failed to find it by making direct plate cultures. As a matter of fact, the typhoid bacillus is found in such a small number of the specimens where we actually know that it is or has been present in the water from which they were obtained, because of cases of typhoid fever which have developed from drinking the water, that we must consider our lack of finding the bacillus in any given case as absolutely no reason for considering the water to be free from danger. Another serious drawback to the value of the examinations 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. The colon bacilli are far more easy to detect, because they are apt to be more abundant, and, also, because they grow more readily in artificial culture media. A method suggested by Theobald Smith is of value in both finding and excluding the presence of bacilli of the colon group. He adds a few drops of the suspected water to glucose nutrient bouillon in fermentation tubes, and keeps it at 37° C. for from thirty-six to forty-eight hours. If no fermentation occurs no colon bacilli are present. If it does occur plates are made and the bacteria isolated and tested. In the bouillon the colon bacilli when present usually increase in numbers and are then readily detected. The

presence of the colon bacilli in water is, except possibly in rare cases, only of importance as an indication of fecal contamination, and, therefore, of the possibility of dangerous infection with other bacteria. Formerly it was considered that its presence indicated human fecal contamination, but we now know that many of the animals contain in their intestines colon bacilli so similar to those found in human beings that we cannot differentiate the one variety from the other; therefore, the finding of colon bacilli in water must always be judged according to the conditions surrounding the water-supply. Thus, it may indicate cattle contamination from the barn or surface water, human contamination, or, in certain conditions, simply the accidental contamination of the stream by wandering cattle or animals. Properly judged, however, the examination for the colon bacilli may yield results of considerable practical importance. Whenever, in water examinations, any special variety of bacteria is found in unusual abundance, the fact should be noted, for sometimes it may be the cause of some prevailing infectious disease; thus the bacillus pyocyaneus has been found in water producing diarrhoea with greenish discharges.

The Obtaining of Water for Examination. Whenever possible the inoculation of the gelatin or agar tubes and the pouring of their contents into the Petri dishes should be done immediately after gathering the samples, otherwise the actual and relative numbers of the different organisms will change. As a rule, the pathogenic bacteria will decrease and the harmless water species will increase. When the plates cannot be made immediately the water held in the sterile vials should be kept nearly at the freezing-point. Even at very low

temperatures some forms of bacteria increase rapidly. Unless the nutrient gelatin or agar inoculations are made within an hour or two the count of the number of colonies is practically useless. Considerable care is necessary in taking the samples of water, so as not to get extraneous organisms in the water from surrounding sources. Three slightly different methods will suffice to indicate how it should be done. A simple and accurate method of collecting the water is to have several graduated sterile glass pipettes plugged

FIG. 35.



Bulb pipette.

FIG. 36.



Graduated pipette.

FIG. 37.

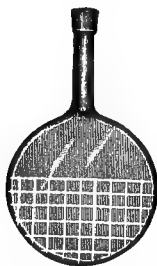


Sternberg bulb.

at the bottom by a cork and above by cotton. This is inserted the required depth, to avoid the surface water with its particles of dirt, and the cork pushed off by a second pipette or rod and the water allowed to flow or be sucked in. The upper end is now stopped by the finger and the pipette removed and a definite amount of water tested (Figs. 35 and 36). A simple glass tube, sterilized by passing it through a flame and corked below, will answer the same purpose, or, again, a tube, one end of which, after sealing, is blown into a sphere and the other end drawn out into a capillary stem

(Fig. 37). The stem must be sealed while the bulb is still hot, or while a little water is being boiled in it, so that a partial vacuum may exist in the bulb, in order that the water may be sucked up into it when the stem is broken. The inoculation of the media is now made directly, or water from the tube is emptied into a sterile bottle or test-tube, or the end of the Sternberg bulb is sealed by heat. When water is to be obtained from greater depths or from beneath the surface of wells, more complicated forms of apparatus are necessary. A good example is the one devised by Abbott,

FIG. 38.



Flask for counting colonies of bacteria.

and made for him by Charles Lentz & Sons, of Philadelphia. It consists of a metal framework in which is encased a bottle provided with a ground-glass stopper. To the stopper a spring clamp is attached, and this in turn is operated by a string, so that when the weighted apparatus is allowed to sink into the stream the stopper may be removed at any depth desired by simply pulling on the string. When the bottle is full the stopper is allowed to spring back into position by releasing the spring. Before removing the water the neck of the bottle should be sterilized by pouring a little of a 5

per cent. solution of carbolic acid upon it and drying with a sterile cloth.

The technique of making plate cultures, of counting the number of colonies, and of isolating and identifying pathogenic species are described under the special chapters devoted to these subjects. A point to be remembered is that about double the number of colonies usually develop at 20° C. as at blood heat (37° C.), many water bacteria not growing at body-temperature. A convenient flat flask with ruled surface (Fig. 38) has been devised to take the place of the Petri dish when the number of bacteria only and not the varieties are wanted. In these there is no danger of contaminating air-organisms entering during transportation. The stopper can be graduated to hold one c.c.

The 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 put either in the incubator at 37° C. or kept at room-temperature. The more careful 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 20° C., according as a growth of the parasitic or saprophytic varieties is desired.

THE CONTAMINATION AND PURIFICATION OF DRINKING WATERS.

Brook and river water is 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, in the course of a few miles, through the dilution of additional supplies, through sedimentation, and through oxidation, 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 a safe water to drink, although with the lapse of time 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 further 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 feces, and six weeks later an alarming epidemic attacked Lawrence, nine miles below Lowell. It was estimated that the water took ten days to pass from Lowell to Lawrence and through the reservoirs. As typhoid bacilli may live for twenty-five days in water, the Lawrence epidemic is easily explained. Newark-on-Trent, England, averaged seventy-five cases a year from filtered water and only ten when it was changed to deep-well supply.

The Purification of Water on a Large Scale. Surface waters, if collected and held in sufficiently large lakes or reservoirs, usually become so clarified by sedimentation 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. Filtration of water exerts a very marked purification, taking out 99 per cent. of the organisms in those best constructed and at least 90 per cent. in those commonly used in cities. The construction of filters is too large a subject to enter on minutely here; they 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 and well waters are, in fact, filtered waters.

Domestic Purification. Water which requires private filtering should not be supplied for drinking purposes. Unhappily, however, it often is. Filters may be divided, roughly, into those for low and high 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 high-pressure 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 class are the Berkefeld and Pasteur filters. These yield a water, if too great 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 simple 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 has filtered. A filter easily made 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.

CHAPTER XVII.

THE CLASSIFICATION OF BACTERIA.

THE PERMANENCE OF VARIETIES.

BACTERIA have been classified in many different ways by many different observers. As a rule, the genera are based upon morphological characters and the species upon biochemical, physiological, or pathogenic properties. While the form, size, and method of division are the most permanent characteristics of bacteria, and so are naturally utilized for classification, nevertheless, in this basis of division there are decided difficulties. Thus while the form and size of bacteria are fairly constant under the same conditions, they are in many quite different under diverse conditions. Another serious drawback is that these morphological characteristics give no indication whatever of the relations of the bacteria to disease and fermentation—the very characteristics for which as physicians we study them. Other properties of bacteria which are fairly constant under uniform conditions are those of spore and capsule formation, motility, reaction to staining reagents, relation to temperature, to oxygen and other food material, and, finally, their relation to fermentation and disease.

Taking any one of these properties of bacteria as a basis, we can classify them; but even here there will be groups which under certain conditions would be

placed in one class and under others in another. Thus the power to produce spores may be totally lost or held in abeyance for a time.

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 given 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 divisions :

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 twice the normal length before the division.
 - (1) Cells straight, rod-shaped, without sheath, non-motile, or motile by means of flagella 2. Bacteriaceæ.
 - (2) Cells crooked, without sheath 3. Spirillaceæ.
 - (3) Cells enclosed in a sheath 4. Chlamydobacteriaceæ.
 - (4) Cells destitute of a sheath, united into threads, motile by means of an undulating membrane 5. Beggiatoaceæ.

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*.

Cell contents without granules of sulphur.

a. Cell threads unbranched.

I. Cell division always only in one plane 1. Streptothrix.

II. Cell division in three planes previous to the formation of conidia.

- 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.

5. *Beggiatoacea*.

Only one species known (*Beggiatoa*, Trev.), which is scarcely separable from *Oscillana*.

A study of the above table will show that it makes changes in the genus of some of the most common bacteria, as in the restoration of the old genus bacterium and the assigning to it of all non-motile, rod-shaped organisms, thus altering the genus of some of the most common pathogenic bacteria from bacillus to bacterium. Other changes are seen in the spirilla. Any such scheme is at times very 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 some authoritative body agrees on some one it is almost useless in such a volume as this to change the usually employed names for others which are, perhaps, intrinsically somewhat 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* a bacterium. We will, therefore, simply use the usual nomenclature, 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.

The Permanence of Bacterial Species. When we come to study special varieties or groups of bacteria, such as the bacilli which produce typhoid fever, diphtheria, 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 can hardly imagine 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 progressed probably exposed certain animals to the invasion of varieties which never before had gained access to them. If the bacteria found the soil suitable, and also some means of transmission to other animals equally susceptible, a pathogenic species became established which at first, perhaps, found conditions only occasionally favorable to it, but later became more parasitic in its characteristics. Thus in some such way a multitude of bacterial groups arose, some of which accustomed themselves to the conditions present in the soil, others to those in fishes, others to those in birds, and others still to those in man.

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 kept practically unaltered. The diphtheria bacilli are the same to-day as when Löffler 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 is true for tuberculosis, smallpox, hydrophobia, leprosy, etc. Under practically unchanged conditions, therefore, as exist in the bodies of men, bacteria which have once become established as parasites continue so long as they remain to retain their peculiar (specific) characteristics. Whether new disease varieties, such as the influenza bacillus, 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 there is no probability that any saprophytic variety now existing can, under any possibility, develop into the now recognized varieties of pathogenic bacteria. It is almost impossible to conceive that any such variety should start with the same characteristics and then develop parasitic tendencies under exactly the same circumstances as those varieties which now produce disease.

Attenuation. It is now a well-established fact that the great majority of parasitic bacteria can be so altered by change of conditions, and especially by being subjected to unfavorable conditions, that they, while morphologically the same, lose their power of developing in the body and of producing specific poisons. When either or both these properties are partially destroyed they can usually be redeveloped; but when power to produce specific toxins is absolutely lost, it is, so far as we now know, lost forever.

The recovery of toxin production is brought about by developing the micro-organism 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 such animals whose resistance has been overcome by reducing their vitality through poisons, heat, cold, etc. Another method is to accustom the micro-organism to the animal's body by letting it remain surrounded by the animal fluids as it rests in a pervious capsule in the peritoneal cavity.

CHAPTER XVIII.

BACILLUS OF TUBERCULOSIS (KOCH'S TUBERCLE BACILLUS).

IT was a common belief many years ago in some countries (kingdom of Naples, 1782) that tuberculosis was an infectious disease; but it is only within comparatively recent times that the infectiousness of tuberculosis has become an established fact in scientific medicine. Villemin (1868) was the first to show experimentally that tuberculosis might be induced in healthy animals and man by inoculations of tuberculous material. Others attempted to microscopically demonstrate the origin of the disease (Zürn, Buhl, Klebs, Toussaint, etc.); but these investigations, though paving the way to the discovery, which it remained for Robert Koch to make, proved to be unsatisfactory and incomplete. The announcement of the discovery of the tubercle bacillus was made by Koch, in March, 1882, at a meeting of the Physiological Society of Berlin. At the same time 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. An innumerable number of investigators now followed Koch into this field, but their observations served only to confirm his original discovery.

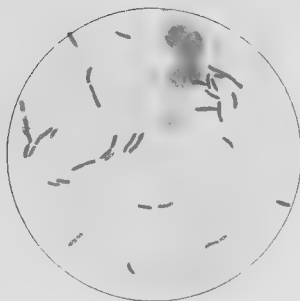
The bacilli are found in the sputum of persons suffering from pulmonary or laryngeal tuberculosis, either free or in the interior of pus-cells; in miliary tubercles and fresh caseous masses in the lungs and elsewhere; in recent tuberculous cavities in the lungs; in tuberculous glands, joints, bones, mucous membranes, and skin affections; in the lungs of cattle suffering from pulmonary tuberculosis, and in tubercular nodules, generally in animals which are infected naturally or by experimental inoculations.

Morphological Characters. The tubercle bacilli are slender, non-motile rods of about 0.2μ in diameter by 1.5 to 4μ in length. (Plate I., Figs. 1, 2, and 3.) 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 forms are observed. In stained preparations there are often seen unstained portions, which have been improperly thought to be spores. From two to six of these unstained spaces may sometimes be noticed in a single rod, and under moderate magnification may give to the bacilli the appearance of short chains of streptococci. In old cultures irregular forms may be obtained, the rods being occasionally swollen at one end or presenting lateral projections.

The *staining* peculiarities of this bacillus are very important, for by them its differentiation and recognition in microscopical preparations of sputum, etc., are rendered possible. 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. Koch first recognized it in a staining preparation to which an alkali had been added—a solution of

PLATE I.

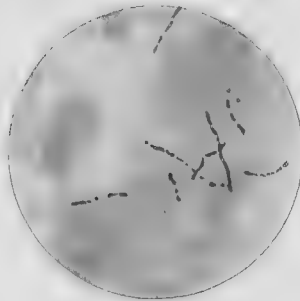
FIG. 1.



Tubercle bacilli, in red.
Strepto-bacilli, in blue.

× 1100 diameters.

FIG. 2.



Tubercle bacilli, in red.
Tissue, in blue.

× 1100 diameters.

FIG. 3.



Very large tubercle bacilli.
Cells in specimen are in blue,
while bacilli are red.

× 1100 diameters.

FIG. 4.



Short smegma bacilli.
Bacilli in specimen are red,
rest of material in blue.

× 1100 diameters.

methylen-blue with caustic potash. More recently Ehrlich devised a method of staining which proved to be better, viz., the use of a solution of an aniline color—fuchsin or methyl-violet—in a saturated aqueous solution of aniline oil and decolorization of other bacteria with a solution of a mineral acid, to be followed by a contrast stain, such as methylene-blue. (Plate I., Figs. 1 and 2.) Various modifications of Ehrlich's method are now commonly used. The carbol-fuchsin solution of Ziehl is largely employed; it has the advantage of acting quickly and keeping well. The tubercle bacilli can be demonstrated also by Gram's method of staining, but this is not recommended for general use.

Biological Characters. The bacillus tuberculosis is a *parasitic, aërobic, non-motile* bacillus, and grows only at a temperature of about 37° C. It has been assumed that this bacillus is capable of forming spores. The refractile spaces, however, are not found to possess the regular shape and brilliancy of ordinary spores, nor have they any greater resisting power to heat, desiccation, etc., than the homogeneous bacilli. Exposure to 60° C. in water destroys them in fifteen minutes. The bacilli have, however, a somewhat greater resisting power than most other pathogenic bacteria, since frequently the bacilli resist desiccation at the ordinary temperatures for months; many bacilli die, however, soon after drying. Portions of the lung from a tuberculous cow, dried and pulverized, produced tuberculosis in guinea-pigs at the end of 102 days (Cadéac and Malet). They retain their vitality for a considerable time in putrefying material. Cold has no effect upon them. When dry the more resistant organisms stand dry heat at 100° C. for hours; but when moist, as in milk, they are more quickly

killed—viz., at 55° C. in one hour, at 60° C. in fifteen minutes, at 65° C. in fifteen minutes, at 70° C. in ten minutes, at 80° C. in five minutes, and at 95° C. in one minute. One reason why they appear to withstand in milk high temperatures for a longer time than given in the above figures is, as pointed out by Theobald Smith, that when heated in a test-tube 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 many bacteria some of them are subjected to less heat than those in the rest of the milk.

The resisting power of the tubercle bacillus against chemical disinfectants is considerable, and is 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 (Baumgarten and others). They are destroyed in sputum in six hours or less by the addition of an equal quantity of a 3 per cent. solution of carbolic acid, and in about one hour by an equal amount of a 5 per cent. solution. Bichloride of mercury is unsuitable for the disinfection of sputum unless used in very strong solutions (1 : 500). From recent experiments by Yersin upon pure cultures of the bacillus it appears that tubercle bacilli were killed by a 5 per cent. solution of carbolic acid in thirty seconds; by 1 per cent. in one minute; absolute alcohol, five minutes; iodoform-ether, 1 per cent., five minutes; mercuric chloride, 1 : 1000 solution, ten minutes. Salting and smoking are said not to destroy the virulence of tuberculous meat (Forster).

The tubercle bacillus 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 seven days when placed near a window. This fact is worthy of note, as it has an important hygienic bearing. Thus, tuberculous sputum expectorated upon sidewalks, etc., being exposed to the action of direct sunlight, will in many cases, especially in summer, be disinfected by the time it is in a condition to be carried into the air as dust. For the same reason, consumptive patients should occupy light, sunny rooms and live as much as possible in the open air and exposed to the action of direct sunlight.

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. But it has been noted that when it is cultivated for a time in artificial media containing glycerin it may grow on the surface of plain veal or chicken bouillon, in which media it fails to develop when introduced directly from a culture originating from the body of an infected animal. This would indicate the possibility of its acquiring the ability to grow as a saprophyte. The experiments of Nutall also show that the bacillus may multiply, under favorable conditions, in tuberculous sputum outside of the body. Notwithstanding these facts, it is probable that the growth of tubercle bacillus outside of the living bodies of man and animals is so slight as to have no practical importance in causing infection.

On account of their slow growth and the special con-

ditions which they require, tubercle bacilli cannot be grown in pure culture by the plate method on the ordinary culture media. Koch first succeeded in cultivating and isolating this bacillus on coagulated blood-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. Roux and Nocard afterward showed that the bacilli from man and animals occasionally grow on nutrient agar to which glycerin has been added in the proportion of 5 per cent.

Growth on Coagulated Blood-serum. On this medium, which is regularly used to obtain the first culture, the growth first becomes visible at the end of ten to fourteen days at 37° C., and at the end of three to four weeks a distinct and characteristic development has occurred. 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. When a tiny piece of this is removed, placed on a cover-glass without rubbing, stained, and then observed under the microscope the surface growth presents a characteristic appearance, the bacilli being arranged in parallel rows of variously curved figures.

Owing to the greater facility of preparing and sterilizing *glycerin-agar*, and the more rapid and abundant growth of the bacilli, which have become accustomed to growth outside the body on this medium, it is now usually employed in preference to blood-serum for preserving cultures. The development at the end of fourteen to twenty-one days is more abundant than upon blood-serum after several weeks. When numer-

ous bacilli have been distributed over the surface of the culture medium, a rather uniform, thick, white layer, which subsequently requires a slightly 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.

Growth on Peptonized Veal or Beef Broth Containing 5 per cent. of Glycerin. 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. The latter of these media is used for the development of tuberculin. 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 on the bottom of the flask, where it ceases to develop further. The liquid remains clear, containing in solution the products formed by the growth of the bacillus, and is really a dilute crude tuberculin. 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.

The Obtaining of Cultures of the Tubercle Bacillus from Sputa and Infected Materials for Diagnostic Purposes. As this is a matter of great and increasing importance, we will consider in detail the methods which have been successfully employed. *Pure cultures* can be obtained directly from tuberculous material; but as it is so difficult to get rid of the other bacteria which are almost always present, and which grow

much more rapidly and take possession of the medium before the tubercle bacillus has had time to form visible colonies, it is best, unless human tissues can be obtained free from other infection, first to inoculate a guinea-pig, both subcutaneously and intraperitoneally, with the sputum, and then obtain cultures from the animal as soon as the tubercle infection has fully developed. From acute tuberculosis in man in other regions than the lungs, where mixed infection usually exists, direct cultures on blood-serum may be made.

The animals thus inoculated usually die at the end of three weeks to four months. It is better, however, to kill a guinea-pig which by its enlarged glands shows evidence of tuberculosis, and to remove, with the greatest care as to cleanliness, one or more nodules from the lungs, spleen, or lymphatic glands. 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 give unsuccessful cultures. The animals after being killed are placed 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, an opening is cut with a fresh set of sterile instruments into the thoracic or abdominal cavity; then with a sterile forceps the lymph-gland portion of spleen or other part which it is desired to examine is removed to a sterile covered beaker. This tissue if suitable may be sliced in thin sections and conveyed directly to the surface of the solid culture medium and gently rubbed over the surface, and then left on it, or a part of it may first be crushed between two sterilized

glass slides and then transferred to the serum and rubbed gently over its surface. Owing to the liability of the blood-serum to become too dry for the development of the bacillus, it is necessary to keep the culture moist by sealing the end in some way, as by applying a rubber cap over the open end of the test-tube, which prevents evaporation. This cap should be sterilized in a solution of mercuric bichloride (1 : 1000) and the end of the cotton plug burned off just before applying it, to destroy any spores of mould fungi present. Theobald Smith, who has had a very large experience in growing the tubercle bacillus, gives the following details as to his method :

“ Throughout the work solidified dog’s serum was used. The dog was bled under chloroform and the blood drawn from a femoral artery, under aseptic conditions, through sterile tubes directly into sterile flasks. The serum was drawn from the clot with sterile pipettes, and either distributed at once into tubes or else stored with 0.25 to 0.3 per cent. chloroform added. The temperature required to produce a sufficiently firm and yet not too hard and dry serum is, for the dog, 75° to 76° C.; for horse and beef serum it is from 4° to 5° lower. The tubes containing the serum were set in a thermostat, into which a dish of water was placed, to forestall any abstraction of moisture from the serum. About three hours suffice for the coagulation. This procedure dispenses with all sterilization excepting that going on during the coagulation of the serum. It prevents the gradual formation of membranes of salts, which, remaining on the surface during coagulation, form a film unsuited for bacteria. Tubes of coagulated serum should be kept in a cold,

closed space, where the opportunities for evaporation are slight. They should always be kept inclined.

“The ordinary cotton-plugged test-tubes I do not use, because of the rapid drying out permitted by them as well as the opportunities for infection with fungi. Instead, a tube is used which has a ground-glass cap fitted over it. This cap contracts into a narrow tube plugged with glass-wool; this plug is not disturbed. The tube is cleaned, filled, and inoculated by removing the cap. With sufficient opportunity for the interchange of air very little evaporation takes place, and contamination of the culture is a very rare occurrence. In inoculating these tubes bits of tissue which include tuberculous foci, especially the most recent, are torn from the organs and transferred to the serum. Very little crushing, if any, is desirable or necessary. I think many failures are due to the often futile attempts to break up firm tubercles. Nor should the bits of tissue be rubbed into the surface, as is sometimes recommended. After a stay of several weeks in the thermostat I usually remove the tubes and stir about the bits of tissue. This frequently is the occasion for a prompt appearance of growth within a week, as it seems to put certain still microscopical colonies in or around the tissues into better condition for further development. The thermostat should be fairly constant, as urged by Koch in his classic monograph; but I look upon moisture as of more importance. If possible a thermostat should be used which is opened only occasionally. Into this a large dish of water is placed, which keeps the space saturated. Ventilation should be restricted to a minimum. As a consequence, moulds grow luxuriantly, and even the gummed labels must be

replaced by pieces of stiff manila paper fastened to the tube with a rubber band. By keeping the tubes inclined no undue amount of condensation of water can collect in the bottom, and the upper portion of the serum remains moist. The only precaution to be applied to prevent infection with moulds is to thoroughly flame the joint between the tube and cap, as well as the plugged end, before opening the tube."

At the Saranac Laboratory beef blood-serum is used in ordinary test-tubes, which are sealed by rubber caps. The tubercles are crushed between sterile glass slides and rubbed gently upon the serum surface. The serum itself must not be too firm. It should just be solid enough to stand upright. The results thus obtained by Trudeau and Baldwin have been as good as those reported by Smith. In our experience all methods frequently fail with those unfamiliar with them, especially when, as shown by microscopical examination, the tubercular tissue used contains very few bacilli.

Pathogenesis. The tubercle bacillus is pathogenic not only to man, but to a large number of animals, such as the monkey, pig, cow, etc. Guinea-pigs are extremely susceptible, and are much used for the detection of tubercle bacilli in suspected material. When inoculated with the minutest doses 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.—are then filled with tubercles. On subcutaneous injection, for instance, into the abdominal wall, there is a thickening of the tissues about the point of inoculation, which breaks down in about a week and leaves 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 always found in the affected tissues, but the more chronic the process the fewer the bacilli that are apt to be present.

Rabbits are also quite susceptible to tuberculosis, but considerably less so than guinea-pigs. In rabbits death almost invariably follows inoculations of tuberculous material into the anterior chamber of the eye. The local effects are iris-tuberculosis and cheesy degeneration of the pupil. The bacilli then penetrate to the neighboring lymph-glands, producing softening of these, then pulmonary tuberculosis, general tuberculosis, and finally

death at the end of several weeks or months. Subcutaneous inoculations are less effective, and in small doses do not always kill. Intravenous and intraperitoneal injections usually produce general tuberculosis and death at the end of a few weeks. The tubercles in rabbits are smaller, as a rule, and the spleen and liver not so much enlarged as in guinea-pigs, but the kidneys not infrequently contain nodules of the size of a pea.

Of other susceptible animals, field-mice and cats are readily infected by artificial inoculations of tuberculous material; rats, white mice, and dogs only when very large doses are given. All these animals present the anatomical lesions of miliary tuberculosis. Bollinger has produced intestinal tuberculosis in calves by inoculating them with material taken from a tuberculous man. Canaries are also susceptible to inoculations of the tubercle bacillus; but not sparrows. Cold-blooded animals of various kinds, according to the experiments of Koch, are immune, unless, as recently demonstrated, the bacilli are first slowly accustomed to growth at low temperatures. Fowls and pigeons are only slightly susceptible to the bacillus derived from man. Among the larger birds, parrots alone would seem to be clearly susceptible.

Beside the artificial modes of infection already referred to, tuberculosis may be caused in animals by *feeding* them with tuberculous material. In this case evidence of infection is usually shown in the mesenteric glands before the intestinal walls are affected. Zagari records some experiments in which tubercle bacilli fed to dogs (one of the less susceptible animals) were absorbed by the mucous membranes of the intestines, and thus reached the internal organs without producing any

local lesions whatever. It would seem to be possible, therefore, that tubercular infection may be caused, under certain conditions, by absorption through serous or mucous membranes without the evidence of any local lesion.

The experimental production of tuberculosis by *inhalation* of bacilli has been demonstrated by Koch in guinea-pigs, rabbits, rats, and mice, and his results have since been confirmed by many others; but in these experiments the bacilli were usually inhaled in the form of a very thin spray in which they were suspended. The experimental inhalation of dry tubercular dust has seldom proved successful.

Various other tubercular affections which are natural in man have been produced experimentally in animals, as, for instance, tuberculosis of the joints (Pawlowsky), tubercular abscess (Courmont), etc.

It need hardly be said that the discovery of the tubercle bacillus has elucidated the etiology of many diseases the origin of which was formerly doubtful. Among these may be mentioned the various forms of tuberculosis of the lungs and other organs, lupus, scrofula, fungoid inflammations of the bones and joints, tuberculosis in cattle, monkeys, horses, swine, sheep, goats, and the so-called spontaneous tuberculosis in guinea-pigs and rabbits in cages in which healthy and artificially infected animals have been kept together.

Of domestic animals cattle are by far the most frequently attacked by this disease. It is also not uncommon in young swine. Monkeys, when they are kept in confinement, die almost invariably from tuberculosis. Among other domestic and wild animals it is a comparatively rare disease. Birds, with the exception of

parrots, are not subject to tuberculosis, and cold-blooded animals are altogether immune.

Beside the affections already referred to in man the following diseases have been traced to tubercular origin: Among skin diseases, so-called inoculation-lupus, tuberculosis-verrucosa cutis, and scrofuloderma; choroidal tuberculosis, idiopathic serous pleurisy and lymphatic enlargements simulating pseudoleukæmia.

The 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, give off sufficient poison to cause the less marked changes only, and never produce cavities (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 the diffuse growth of a tissue identical in structure with that of miliary tubercles—that is, composed of a basement substance containing epithelioid, giant, and lymphoid cells. This diffuse tubercle-tissue also tends to undergo cheesy degeneration.

Distribution of Tubercle Bacilli in the Tissues. In acute tuberculosis, especially when caseation is rapidly spreading, the bacilli are usually abundant. They are generally scattered irregularly through the tissues or in small groups. They are occasionally found in the leucocytes and in the giant and epithelioid cells. In subacute and chronic lesions they are usually few in number. Sometimes in old caseous materials numerous stained granular points are seen; these are supposed by some to be a resting stage similar to spores.

Infection. Infection by the tubercle bacillus takes place usually through the respiratory tract or the digestive tract, more rarely through wounds of the skin.

In the majority of cases the mode of infection is evident. Pulmonary tuberculosis as a primary disease, and not occurring in young children, may be considered to be caused chiefly by the direct transmission of tubercle bacilli through kissing, soiled hands, handkerchiefs, etc., or by the inhalation of tuberculous dust. Intestinal and mesenteric tuberculosis, which is rare among adults and common with children, is probably due not only to swallowing the bacilli received in the above ways, but also to the ingestion of tuberculous milk. Lupus is probably always produced by the inoculation of tubercle bacilli on the skin or mucous membranes, which is indicated by the fact that the original seat of the disease is so often on a wounded

surface. Localized skin tuberculosis is sometimes produced by inoculation at autopsies.

Infection by Inhalation of Tuberculous Dust. Certainly one of the common modes of infection is by means of tuberculous sputum, which, being coughed up by consumptives and carelessly expectorated, dries and 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 only when it becomes dry, as on handkerchiefs, bedclothes, and the floor, etc., that the dust is a source of danger for infection. 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 from one-half to three billion virulent tubercle bacilli (according to the experiments of Nutall) 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. There is comparatively little danger of infection at a distance, as in the streets, for instance, where the tubercle bacilli, even if present in the dust, have become so diluted that they are not much to be feared. Exhaustive experiments made by many observers have shown that particles of dust collected from the immediate neighborhood of consumptives, when inoculated into guinea-pigs,

produced tuberculosis in a considerable percentage of them; whereas the dust from rooms inhabited by healthy persons or the dust of the streets did 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 practically free from living pathogenic bacteria. It is the coarser particles in which the bacilli are protected by an envelope of mucus that resist drying for considerable periods. These are carried only short distances by air currents. Such results as those obtained by Straus, who, 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 derived from grasses which look and stain like tubercle bacilli and yet are totally different. It has been argued by some, from the fact that about one-seventh of all men die from tuberculosis, that the tubercle bacilli must be ubiquitous, and that precautions are useless; but, as Cornet has pointed out, this does not mean that one-seventh of all men living are tuberculous, for no man is tuberculous during the entire course of his life, but only for a limited period (variously estimated at from three to eight years). It may, therefore, be said that the danger of infection from tuberculosis in general is not so great after all, but that on this account it is all the more to be feared and guarded against in the immediate neighborhood of consumptives. Those who are most liable to infection from this source are the families, 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. Flügge has recently drawn attention to the fact that in coughing, sneezing, etc., very fine particles of throat secretion are thrown out and carried by air currents many feet from the patient and remain suspended in the air for a considerable time. This is another means of infection, but probably an infrequent one. We have now to encourage us a mass of facts which go to show that when the sputa is carefully looked after there is very little danger of the infection of others except by close personal contact.

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. Whatever may be the opinion with regard to these outward signs, there is no doubt that personal susceptibility is of the greatest importance in the production of this disease. Unquestionably, vast differences exist in different individuals in the intensity of the tubercular 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 in the tubercle bacilli obtained from different sources. As is seen from the results of post-mortem examinations in which the remains of old tubercular processes have been found in the lungs of about one-third of all the bodies examined, many cases of pulmonary phthisis must occur without showing any visible evidences 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 poor hygienic conditions, depressing surroundings (as in asylums and prisons), obstinate bronchial affections, diabetes, and other exhausting diseases increase the susceptibility 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. This is not so evident among guinea-pigs, which are so susceptible that they succumb to an inoculation of the minutest dose of virulent bacilli; but rabbits are not always killed by subcutaneous inoculations, though some individuals die from very small doses. Dogs, rats, and other more resistant animals show this still more plainly. Man cannot be placed on the same plane of susceptibility to tuberculosis with guinea-pigs, for with him the disease often remains local or is entirely cured. The doctrine of individual susceptibility, therefore, is seen to be founded on fact, although the reasons for it are only partially understood.

Infection by Ingestion of Milk and Meat. Phthisical sputum, however, is not held responsible for the occurrence of all human tuberculosis. Milk also serves as a conveyer of infection, whether it be the milk of nursing mothers suffering from consumption or the milk of tuberculous cows. The transmission of tubercle bacilli in the milk of tuberculous individuals has only been indirectly established in human beings, but in cow's milk it has been abundantly proved. Formerly it was thought that in order to produce infection by milk there must be local tubercular affection of the udder; but it is known now that tubercle bacilli may be found in the milk when an internal organ is infected and when careful search fails to detect any udder disease. So that the milk of every cow which has any internal tubercular infection must be considered as possibly containing tubercle bacilli. Rabinowitsch and Kempner proved beyond all question that not only the milk of tubercular 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 the milk of from 20 to 60 per cent. of tuberculous cows. When we consider the prevalence of tuberculosis among cattle we can readily realize, if the bovine bacillus readily infects human beings, the danger to which children are exposed from this source of infection. Thus, taking the abattoir statistics of various countries, we find that in Prussia 8.3 per cent. of the cattle slaughtered were tuberculous; in Dresden, 14.4 per cent.; in London, 25 per cent.; in Berlin, 12 per cent.; in New York, about 7 per cent. Another possible source of infection in intestinal tuberculosis is

the flesh of tuberculous cattle. Here the same conditions hold good as in the infection by milk, only 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 great mortality from tubercular diseases among mankind, 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, especially among children, the sterilization (by heat) of the milk used as food must commend itself to all. It is only right to state, however, that the actual proof that human tuberculosis has come from milk or food infected with bovine tuberculosis is very small, and that it is perfectly possible that the bovine bacilli may not be as virulent for man as for animals, still we know that human tuberculosis produces bovine tuberculosis in young and susceptible animals, and the reverse is in all probability true. The relation of bovine to human tubercle bacilli will be discussed later in this chapter.

Auto-infection by Swallowing Sputum. The *secondary* forms of tuberculosis which often succeed a primary infection of the lungs may be explained as an auto-infection from the swallowing of sputum containing bacilli, these passing through the gastric juice unaffected. It is a wonder, indeed, that intestinal tuberculosis is not more common than it is in consumption; but this is probably due to the fact that in adults the intestines are comparatively insusceptible. Tuberculosis may also begin as a local infection in the lungs or intestines, and thence extend to other parts of the body, until, passing into the circulation, a general miliary tuberculosis results.

Hypothesis of Transmissibility of Tubercle Bacilli to the Fœtus. Baumgarten and others have advanced a hypothesis to account for certain obscure cases of tuberculosis—namely, that of the transmissibility of tubercle bacilli from the mother to the unborn babe. There seems to be some evidence of the possible transmission of tubercular poison from the mother to the fœtus in animals. The first authentic case recorded is that reported by Johne of an eight-months-old calf fœtus; other cases have since been reported. With regard to tuberculosis in the human fœtus the evidence is not so clear, though several cases have been reported of tuberculosis in very young babies only a few weeks old, and two cases are recorded of placental tuberculosis. The fact that statistics show a greater frequency of tubercular diseases in children during the first than in the following years of life does not strengthen the hypothesis of infection *in utero*; for nursing babies would naturally be more exposed to infection through the mother's milk and through personal contact than others, and, beside, the more tender the life of the infant the more susceptible it would be ordinarily to indirect infection from a tuberculous mother. Experimental proof, however, of the actual transmission of tubercle bacilli from the mother to the fœtus in animals has recently been furnished. De Rienzi found that in five out of eighteen cases in guinea-pigs such transmission of bacilli did take place, and Gärtner confirmed the same in numerous experiments on rabbits, mice, and canaries. The infection resulted not only from animals affected with general miliary tuberculosis, but also from local disease of the lungs; but in the majority of cases very few bacilli were transmitted to the fœtus—so few,

indeed, that it required the inoculation of the entire contents of the body to cause tuberculosis in guinea-pigs; moreover, only one or two of a litter were affected at one time. According to these experiments one would expect to find in man foetal or placental tubercular infection more common than it is, whereas it is extremely rare, even if the few cases reported be accepted as proven. Possibly the few bacilli which may be transmitted to the foetus do not find conditions favorable for their development, and, being so few in number, die; or they may remain latent, as has been suggested, for certain lengths of time without producing visible effects, and only show symptoms of infection later; but we have no experimental confirmation of any such latency existing with regard to the tubercle bacillus, and it is not to be assumed that it does exist. As to the infection of the foetus from the paternal side, where the father has tuberculosis of the scrotum or seminal vessels (which have been found to be tuberculous in exceptional cases), we have no reason to suppose that such can occur. There are, however, some 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.

Length of Time Tubercle Bacilli Remain Virulent in Sputum. Of considerable importance in studying the subject of tubercular infection is the question of the

length of time during which the tubercle bacillus retains its virulence, and whether there are any naturally attenuated varieties. According to experimental investigations, the virulence of dried tubercular sputum is not suddenly but gradually lost, a certain proportion of it retaining its specific infective power under ordinary conditions, as in a dwelling-room, for at least two or three months. An instance is reported by Ducor (Paris, 1890) of a healthy family having been infected with tuberculosis from living in a room which had been occupied by a consumptive two years before, and on examining the sputum-stained wall-paper not only were tubercle bacilli found in it, but upon being inoculated into guinea-pigs they died of tuberculosis.

Attenuation. Metschnikoff states that when kept at a temperature of 42° C. for some time the tubercle bacillus undergoes a notable diminution in its pathogenic power, and that when kept at a temperature of 43° to 44° C. it after a time only induces a local abscess when injected subcutaneously into guinea-pigs. The experiments of Lôte also indicate that an attenuation of virulence has occurred in the cultures preserved in Koch's laboratory, originating in 1882 from the lungs of a tuberculous ape. A culture of ours which we obtained from Trudeau, and which has grown now either at Saranac or in our laboratory for six years, is no longer capable of causing tuberculosis in guinea-pigs, although originally virulent.

Mixed Infection. Some time ago attention was drawn to the fact that tuberculosis, whether of the lungs, lymphatics, or cold abscesses, was often a mixed infection. The other micro-organisms with which the tubercle bacillus is most commonly associated 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, and not infrequently on their invasion the disease takes on a septicæmic character. For the technique employed in examining sputa for mixed infection, see later in this chapter.

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 unsatisfactory. Among the numerous medicinal agents that have been tried 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. Various inoculation experiments with cultures of the tubercle bacilli and their products have been made, and though the results reported in some cases have been temporarily favorable, immunization has never been satisfactorily produced.

Koch's Tuberculin. The discovery by Koch of toxins in cultures of the tubercle bacillus which possess properties which explain its pathogenic power must rank as one of the first importance in scientific medicine, on account of what it has led up to, even if—as appears probable—the final verdict may be that its therapeutic value in the treatment of tubercular diseases in man is very slight.

Tuberculin contains all the products of the growth of the tubercle bacilli in the nutrient bouillon as well as some substances extracted from the bodies of the

bacilli themselves. It also contains all the albuminoid and other materials originally contained in the bouillon which have remained unaffected by the activities of the bacilli. There are two preparations known respectively as the old and the new tuberculin.

Old 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 4 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 microscopical examination, are passed into a suitable vessel and evaporated to one-tenth of their original bulk over a water-bath at a temperature of 70° to 80° C. The liquid is then filtered through chemically pure sterilized filter-paper. The crude tuberculin thus obtained contains 40 to 50 per cent. of glycerin and keeps well, retaining its activity indefinitely.

The method of treatment and the results obtained from the old tuberculin have been described recently by Koch briefly as follows: After each injection, which should be large enough to cause a slight but not a great

rise of temperature, a noticeable improvement in the tuberculous process results. The amount of tuberculin injected is continually increased, so as to continue the moderate reactions. After several months all reactions cease, the patients having become temporarily immune to the toxin, but not to the growth of the bacillus. Further injections are now useless until this immunity has passed. During the treatment the bacilli themselves have not been directly affected, and when the treatment is interrupted the tuberculous process is apt to progress. Many cases, however, of pure tuberculosis become cured or greatly benefited by several periods of treatment.

The substances produced in the body by the old tuberculin neutralized the tubercular toxins, according to Koch, but were 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 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. Büchner 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.

The *new tuberculin* formed by either of these methods is a watery extract of the soluble portions of the unaltered tubercle bacilli. As can be readily seen, in a preparation thus made contamination is difficult to avoid, freedom from intact bacilli is uncertain, and the strength of the solution prepared at different times is variable. Twenty per cent. of glycerin is added to preserve the tuberculin from contamination. After three years of trial the results obtained with the new tuberculin preparations cannot be considered to have exerted either very different or very superior effects to the older product.

As to the results obtained in general the reports are as yet conflicting. Lupus seems to be decidedly benefited for a time both by the old and the new tuberculin. Relapses are, however, common. On advanced phthisis, laryngeal tuberculosis, and other tubercular processes no effects have been noted, and nearly every one disapproves of their use in these cases as well as in those where mixed infection is suspected; even in cases of beginning infection, opinions, as a whole, are not very enthusiastic. The new tuberculin is, except when prepared with the utmost care, a dangerous substance, for Trudeau, Baldwin and others found that guinea-pigs injected with it not only did not become immunized, but actually became infected from the living bacilli in the fluid.

The chief use to which tuberculin has been put is as an aid to the diagnosis of tuberculosis in cattle and human beings, 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 centi-

grammes produces in at least 95 per cent. a rise of temperature of from 1° to 3° C. The febrile reaction occurs in from twelve to fifteen hours after the injection. Its intensity and duration do not depend upon the extent of the tuberculous lesions, but is 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 occurs from the subcutaneous injection of the dose mentioned. For these injections the crude old tuberculin is used, which for the convenience of administration is diluted with water. The following are the 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 unusual 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 the tuberculin in the evening, preferably between the hours of six and nine. The injection should be made with a carefully sterilized hypodermatic 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 hour after the injection.

“(d) When there is no elevation of temperature at this time (sixteen hours after the injection) 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 reaction is detected prior to the sixteenth hour, the measurements of temperature should be continued until the expiration of this period.

“(f) If there is an unusual change of temperature of the stable, or a sudden change in the weather, this fact should be recorded on the report-blank.

“(g) 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.

“(h) 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.

“(i) The tuberculin test is not recommended for calves under three months old.

“(j) In old, emaciated animals and in re-tests use twice the usual dose of tuberculin, for these animals are less sensitive.

“(k) Condemned cattle must be removed from the herd and kept away from those that are healthy.

“(l) In making post-mortems the carcasses should be thoroughly inspected, and all of the organs should be examined.”

Tuberculin injections are also made in man to reveal a suspected tuberculosis. 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 at all the extent of the disease, nor whether the tuberculosis is active or dormant. 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. I quote here Dr. Trudeau upon the use of the test.

“In the absence of any well-defined rules founded upon the experience of others at the time I began to use the test, the method I adopted has been a purely arbitrary one, and I make no claim for its being the best or the most reliable, although, as far as my own personal experience goes, I have as yet seen no objection to it or any reason to modify it.

“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., 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 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 further, 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 surroundings 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 and indicate the existence of some slight latent tuberculous lesion, and the test 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. The minute amounts adopted by Grasset and Vedel—*i. e.*, from 0.0002 to 0.0005—while they have the advantage of absolute safety, may lead into error, as they are insufficient, on the evidence of these observers themselves, to cause reaction in cases proven to be tuberculous by the presence of the bacillus in the expectoration. If, on the other hand, the test be pushed to the injection of such large amounts as 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."

Antituberculous Serum. Whether serum-therapy is destined to solve the problem of the treatment of tuberculosis remains for the future to decide, but up to the present the results obtained with antituberculous serum do not warrant our forming such an opinion. The attempts to obtain from animals—chiefly horses—a serum which would be protective have been carried out along very much the same lines as Koch's experiments upon man. The methods adopted have been as follows: Old cultures of tubercle bacilli grown in 5 per cent. glycerin bouillon have been filtered either with or without previous boiling, and then injected into animals, this process being similar to Koch's with his first tuberculin. Others have injected living virulent or non-virulent tubercle bacilli, either alone or with their culture fluids; others still (Büchner) have injected the bacterial protoplasm obtained by crushing tubercle bacilli together with sand and squeezing them; this, like Koch with his new tuberculin, being an attempt to get from the unaltered products and cell-contents of the bacilli the formation in the body of bactericidal or immunizing substances.

Among the many claiming good results in man or animals thus treated may be mentioned Hericourt, Richet, Bernheim, Maragliano, Viquerat, Paquin, de Schweinitz and Dorset, McFarland, and others. The majority claim for their serum the power to neu-

tralize the effect of tuberculin when injected into tuberculous guinea-pigs; but this test is insufficient and probably valueless, since tuberculin is not the same as the unaltered products of the tubercle bacillus. Moreover, it has been shown by Trudeau and Baldwin that other substances which have no specific properties whatever will have much the same effect as the serum under certain conditions. Some make the further claim that guinea-pigs injected with serum acquire an immunity to the virulent tubercle bacilli, and that those already infected live longer than the controls which receive no serum; and some even claim to be able to cure animals eighteen days after inoculation with a culture of tubercle bacilli. Very few observers, however, have succeeded in obtaining appreciable results with the serums prepared by other experimenters. In spite of such conflicting testimony, it is probably safe to assert that no serums 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.

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 condition of the people in general, we should endeavor to build up the individual resistance to the disease. It may be years yet before the public are sufficiently educated to co-operate 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.

The Tubercle Bacillus of Cattle 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. The bovine bacillus, as the most important of the group, will be alone considered here.

The bacilli derived from cattle are on the average a little shorter and straighter than the average human bacillus; but there are many derived from cattle exactly similar to those derived from man in size, shape, and staining. In guinea-pigs, and especially in rabbits, the bovine bacilli are more virulent than those from human sources. Animals infected with the bacilli from cattle, as well as those from the other domestic animals, react to the tuberculin test. All these bacilli are, therefore, undoubtedly from the same original stock, and at first glance we might consider it unnecessary to prove that those derived from cattle were capable of causing human tuberculosis. There are facts, however, which tend to make us doubtful of the extent to which this infection takes place. As we investigate we find that

all facts tend to show that the great majority of cases, in adults at least, come from human infection. The cases where fairly strong proof of bovine infection has been obtained are certainly rare.

Further, we have the undoubted fact that constant sojourn in one species of animal tends to increase the virulence of the germ for that animal and to lessen it for others.

Theobald Smith has made the interesting discovery that there is a wide difference between the culture growth of the average bovine bacillus and the average one from human sources, the bovine bacilli being shorter and straighter, and growing less luxuriently than those from man ; and, further, that the bovine bacilli are much more virulent for rabbits. He has found these differences persist for long periods, and believes that the simple passage through a single person in a case of human tuberculosis would not be sufficient to change these characteristics. He has not yet had a chance to examine the bacilli of any case in young children where milk infection was strongly suspected, but in adults not one of some half a dozen cultures showed the bovine characteristics.

At present it seems fair to assume that bovine bacilli are capable of infecting only those that are very susceptible, such as young children. This question is in great need of further study, and unless proof is brought to show that bovine bacilli never infect human beings, no cattle which are shown to be tubercular should be allowed to furnish milk, or at least none unsterilized should be used for drinking purposes. The flesh is less harmful, as muscular tissue is seldom infected.

Bird (Avian) Tuberculosis. Tuberculosis is very com-

mon and infectious among fowls. The bacilli themselves grow more readily on artificial culture media and produce a more even and moist growth. The bacilli are more apt to show branching forms than the human. In rabbits they produce very similar lesions. They are probably from the same stock as the mammalian varieties; but it is not believed that they are any, and certainly not any great, factor in the production of human tuberculosis.

Diagnosis. 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 render it possible by the bacteriological examination of microscopical 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 microscopical examination. For the animal test, however, time is required—at least three weeks, and, when the result is negative, several months—before any positive conclusion can be reached, for when only a few bacilli are present tuberculosis develops slowly in animals.

LABORATORY TECHNIQUE IN THE EXAMINATION FOR TUBERCLE BACILLI AND OTHER ASSOCIATED BACTERIA.

I. Microscopical 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 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 occurred, 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 entirely vitiate the result, it is best to examine it in as fresh a condition as possible.

2. **Methods of Examination.** (a) **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 one of the small, white or yellowish-white, cheesy masses or “balls” which it is seen to contain. From this make a cover-glass “smear” in the usual way. Immerse this in a *solution of Ehrlich’s aniline-water fuchsin* (see page 198), contained in a thin watch-glass or porcelain dish, and steam over a small flame for two minutes. Then remove the cover-glass from this and wash with water. Now decolorize by immersing the stained preparation in a 3 per cent. hydrochloric acid solution in

alcohol for from a few seconds up to one minute, removing at the time when all color is just about gone from the cover-glass smear. Wash thoroughly with water and make a contrast stain by applying a cold solution of Löffler's alkaline methylene-blue—

Concentrated alcoholic solution of methyl blue 30 c.c.

Caustic potash (1:10,000 solution) . . . 100 “

for from fifteen to thirty seconds. Wash with water; press between folds of filter-paper; dry in the 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 II., 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 seems unnecessary to substitute others for it. 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.

Instead of the Koch-Ehrlich aniline-water solution, *Ziehl's carbol-fuchsin solution* may be used, and is by many preferred (see page 198). Instead of floating the cover-glass smears on the staining fluid they can be

held in the Cornet forceps, covered and kept covered completely with fluid while steamed for two minutes over the flame.

The Koch-Ehrlich 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 cover-glass "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 grammes.
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 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. Ribbert proposed the addition to the sputum of a 2 per cent. solution of caustic potash and boiling the mixture. The mucus is dissolved, and when the mixture is placed in a conical glass vessel any bacilli present are deposited at the bottom, and may be found in the sediment after removing the supernatant

fluid. The sedimentation may be obtained more quickly by the centrifugal machine.

II. 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 equal importance, both for purposes of prognosis and treatment, that the presence of other micro-organisms 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 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 and with the influenza and pneumonia bacilli have also been frequently met with by us. The tetragenus has not been detected by us in thoroughly washed fresh sputum, but has been found by others. At present the facts seem to prove that the tubercle bacilli have in the great majority of cases at least until shortly

before death, a more important rôle than the associated bacteria.

The great majority of stained smears from specimens of sputa show not only the tubercle bacilli stained in red, but many other bacteria stained blue. Some of these associated bacteria have come from the diseased areas of the lungs, while others were merely added to the sputa as it passed through the mouth, or have developed after gathering. To separate the one from the other we wash the sputa.

Sputum Washing. The first essential is that the material be washed within a few minutes, and certainly within an hour, of 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.

If it is desired to examine the specimens for capsule bacteria, pneumococci, etc., they may be stained by Welch's acetic-acid method (page 203) or by Gram's method (page 203).

When we wish to thoroughly 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 Petrie 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 micro-organisms.

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

forty 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 which appeared to be 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 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 Microscopical Examination of Sputum.

Always make two cover-glass preparations from each specimen. Report no result as negative until at least two preparations have been subjected to a thorough search with a 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 that 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 later appear. 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 proves 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 injections of tuberculin can be made use of.

III. 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.

The Ehrlich's Method. Place the paraffin sections in aniline fuchsin and leave for from one to twelve hours; then decolorize by placing them for about half a minute in dilute nitric acid (10 per cent.); wash in 60 per cent. alcohol until no more color is given off; counter stain for two or three minutes in a saturated aqueous solution of methylene-blue, wash in water; dehydrate with absolute alcohol; clear up 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.

CHAPTER XIX.

BACILLI SHOWING SIMILAR STAINING REACTIONS TO
THOSE OF THE TUBERCLE BACILLI — SYPHILIS
BACILLUS—SMEGMA BACILLUS—LEPROSY BACIL-
LUS—GRASS BACILLI.

SYPHILIS BACILLUS.

DISCOVERED by Lustgarten in syphilitic lesions and secretions of 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. 39, page 313.)

Morphology. Straight or curved bacilli, which bear considerable resemblance to tubercle bacilli, but differ from them in staining reactions. They are from 3 to 5μ long and from 0.2 to 0.3μ broad, usually curved or bent at a sharp angle, or S-shaped, often thickened at one end and irregularly notched. With a high-power lens bright, shining spaces in the deeply stained rods may be observed; these, from two to four in a single rod, are believed by Lustgarten to be spores. 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.

The bacillus of Lustgarten *stains* with equal difficulty as the tubercle bacillus, but is much less resistant to the action of certain decolorizing agents, such as mineral acids, particularly sulphuric acid. It is, as a rule, more resistant to the decolorizing action of alcohol than the smegma bacillus.

Biological and Pathogenic Properties. Numerous attempts have been made to cultivate the bacillus of Lustgarten on artificial media, but without success. The inoculation of animals with syphilitic tissues and secretions has also given only negative results, though in man, as is well known, such inoculation has often taken place, the tertiary products only being non-infectious; but as the bacillus has never been obtained in pure culture, we have no positive information as to its biological characters or pathogenesis.

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, but the failure to find other micro-organisms, and the occurrence of these characteristic bacilli in various parts of the body, would seem to point to their etiological importance; while, on the other hand, the long immunity in syphilis, so different from that in any known bacterial disease, casts doubt not only on the status of this bacillus, but also upon the bacterial nature of the micro-organism. The fact that the bacilli have been found occasionally in tertiary lesions—which, however, are known to possess no infectious property—may possibly be explained by the some-

what improbable assumption that the bacilli here present have become attenuated or have died. The finding of saprophytic bacilli—the so-called smegma bacilli—(Fig. 39 and Plate I., Fig. 4), almost identical

FIG. 39.



Smegma bacilli, similar in appearance to syphilis bacilli. $\times 1000$ diam.

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 quickly decolorized by alcohol, but quite resistant to 5 per cent. sulphuric acid solution. Beside, the syphilis bacillus has been found in papules, in gummata, and other syphilomata where there seems no probability whatever of the smegma bacillus having emigrated. Baumgarten, who has searched in vain for Lustgarten's bacillus in uncomplicated visceral syphilo-

mata, suggests that the bacilli found in such lesions were, perhaps, tubercle bacilli, and represented a mixed infection. This may have been true of some cases, no doubt, as the differentiation of new-growths of tertiary syphilis and tuberculosis is often difficult; but the differentiation of the two bacilli can usually be made by their different powers of resistance to the decolorizing action of acids. Finally, other micro-organisms have been described and claimed to be the specific cause of syphilis, but none of these discoveries have been confirmed. From this it appears that though there is no conclusive proof of the fact, there is some possibility, but hardly a probability, that Lustgarten's bacillus is the true cause of syphilis. Its position at present is too doubtful to make its detection of any diagnostic value.

Syphilitic Infection. Infection of those not immune can take place at any time when an abrasion, however small, is brought in contact with the blood or secretions from the primary or secondary lesions of syphilitics.

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 bacillus 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 tuber-

culosis 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 carbolfuchsin, 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 the smegma bacillus. If it is still not decolorized, 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.

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 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. Thus double-stained preparations may be made by first staining sections or cover-glass preparations in Ziehl's carbol-fuchsin solution or in an aqueous solution of methyl-violet, decolorizing in acid, washing in alcohol, and counter-staining with methylene-blue or fuchsin.

Biological Characters. Attempts to cultivate the bacillus lepræ have been frequently made, but so far with only questionable results. None of the cultures obtained have given positive results when inoculated into animals.

Pathogenesis. Numerous inoculation experiments have been made on animals with portions of leprous tubercles, excised for the purpose from lepers, but although a few positive results have been reported, 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 entirely upon the demonstration of its constant presence in leprous tissues.

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, and the mucous membranes of the mouth, gums, and larynx, and in the interstitial processes of the nerves, the 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 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.

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 lepræ occurs also in the blood, partly free and partly within the leucocytes, especially during the febrile stage which precedes the breaking out of fresh tubercles (Walters and Doutrelepon). 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 intra-uterine life seems to have been 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 inoculated a condemned criminal in the Sandwich Islands with fresh leprous tubercles, and which has been generally regarded as positive evidence

of the transmissibility of the disease in this way, is 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 tubercular tissue are mostly dead, or much more probably that an individual susceptibility to the disease is requisite for its production.

The wide-spread 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 if the observation recently made is true, that leprosy reacts, both locally and generally, to an injection of tuberculin in the same manner as tuberculosis (Babes and Kalindero).

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 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, however, are 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 injected animals are unaffected by tuberculin injections. The chief interest which these bacilli have for us is the possibility of confusing them with the tubercle bacilli. This danger is always present in milk, for the grass bacilli find so many means of gaining entrance to it. In the examination of dust, healthy throat and nose secretions, etc., the simple microscopical examination might lead to error.

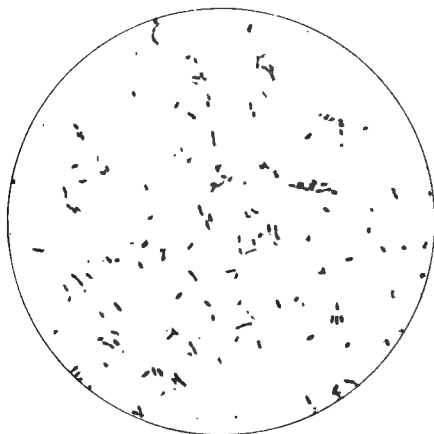
They can be separated from tubercle bacilli by inoculating animals, and then if they show any infection, injecting tuberculin, when if infected with tuberculosis they will die, but if by grass bacilli they will show no reaction. Cultures from the lesions will also show on ordinary media pink colonies if grass bacilli are present, and no growth if only tubercle bacilli.

CHAPTER XX.

INFLUENZA BACILLUS.

AFTER numerous unsuccessful attempts during the epidemic of 1889 and succeeding years to discover the specific cause of influenza, Pfeiffer succeeded in isolating a bacillus (1892) from the purulent bronchial secretion of patients suffering from epidemic influenza which he

FIG. 40.



Influenza bacilli. $\times 1100$ diameters.

showed was the probable cause of the disease. This discovery has since been confirmed by many observers, the results of whose researches give us reason to believe that this bacillus is the chief etiological factor in the production of influenza.

Morphology. Very small, moderately thick bacilli, usually occurring singly or united in pairs, but threads or chains of three, four, or more elements, are occasionally found.

The bacillus *stains* with difficulty with the ordinary aniline colors—best with dilute Ziehl's solution or Löffler's methylene-blue solution, with heat. When faintly stained the two ends of the bacilli are sometimes more deeply stained than the middle portion. Those we have examined, all obtained from cases in New York, were not stained by Gram's method, but some report instances in which they were.

Biological Characters. An *aërobic*, non-motile bacillus; does not form spores; no growth occurs below 26° C., or above 43° C., or in the entire absence of oxygen. This bacillus is best cultivated at 37° C., and on the surface of the ordinary nutrient culture media containing hæmoglobin or purulent material. Plain or *glycerin-agar*, or *blood-serum* streaked with sputum, pus, or blood, make a good soil for their growth. At the end of eighteen to twenty-four hours in the incubator very small, drop-like 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 in the ice-chest.

The Detection of the Influenza Bacillus in Sputum.
When it is desired to obtain cultures of the bacillus of influenza for diagnostic purposes from material suspected to contain this organism, it is advisable from the start to make use of plate cultures, the best medium being nutrient agar freshly smeared with rabbit's blood. The sputum, blood, or other substance to be examined is streaked across several plates of blood-smeared agar, so as to leave on some considerable of the material and on others merely the slightest trace. An easy way to get blood when a large number of plates are to be made is to kill a rabbit and autopsy it immediately. The skin is turned back from the chest and the thorax opened aseptically. The heart is cut off at its base and dragged over some twenty to forty plates, as desired. The blood collecting in the thorax is used to smear the agar in a number of tubes, which can be kept in the ice-chest until needed. With a little skill blood can be withdrawn aseptically from the ear vein of a rabbit by means of a glass tube armed with a hypodermic needle.

When cultures are made from sputa the endeavor should be made to collect the expectoration which comes up naturally, so as not to get any more than necessary of the mouth bacteria. If the mouth is at all foul, it should be cleansed before gathering the sputum. Cultures should be made as soon as possible after obtaining the material. The plates are put in the incubator for

eighteen hours and then examined under a magnification of about 100 diameters. The influenza colonies, when present, will be found in the neighborhood of the blood-cells, much lighter in hue, somewhat smaller, and more finely granular than those of the pneumococci. They appear scarcely more noticeable than the groups of blood-cells that have lost their color and largely disintegrated. With higher magnification the colonies do not show the individual bacteria distinctly, and thus contrast with the pneumococci. The suspicious colonies are fished out, inoculated upon blood and simple nutrient agar, and examined microscopically. When the detection of the bacilli is important, and there are any purulent masses in the sputum, as in influenza complicating phthisis, these are washed, as under directions for examination of sputa for mixed infection (page 306).

On 1.5 per cent. sugar-agar growth also occurs, the colonies appearing as extremely small droplets, clear as water, often only recognizable with a lens (Pfeiffer). In *bouillon* a very scanty development takes place, unless blood is added. At the end of twenty-four hours small, white particles are seen on the surface, which subsequently sink to the bottom, forming a white, woolly deposit, while the *bouillon* remains clear.

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, but soon dies in water. The thermal death-point is 60° C. with five minutes' exposure (Pfeiffer and Beck). In *bouillon* cultures and in sputum at 20° C. they retain

their vitality for from a few days to two or three weeks.

Pathogenesis. The bacillus of influenza, in so far as experiments show, produces the disease only in monkeys and to a less extent in rabbits. From numerous experiments made by Pfeiffer on guinea-pigs, rats, mice, and pigeons these animals seem to be more or less insusceptible to influenza. 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 that 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. 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, and in one case an abscess was produced from an injection into the subcutaneous intercellular tissues; but in no instance has Pfeiffer observed a multiplication of the bacilli introduced. Recently Cantani has shown that it is possible to produce an infection of influenza in rabbits when inoculated with small doses ($\frac{1}{4}$ to $\frac{1}{2}$ c.c.) of living bacilli, provided the point of least

resistance is chosen for the inoculation—viz., the brain, upon which the toxic products of the bacillus influenzae acts most powerfully.

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.

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 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. Very frequently, perhaps almost invariably (Finkler), 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 almost identical with bronchopneumonia due to the pneumococcus. The walls of the bronchioles and alveolar septa become densely infiltrated with leucocytes, and the lumina of the bronchial tubes and alveoli are similarly filled. The pus-cells are found to contain more or less influenza bacilli. 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 purulent exudation. The pleurisy which follows influenza, however, is usually a secondary infection, due to the streptococcus or pneumococcus. Ordinarily influenza runs an acute or subacute course, and not infrequently it is accompanied by mixed infections, with the pneumococcus and the streptococcus. Pfeiffer was the first to draw attention to certain chronic conditions depending upon the influenza bacillus. According to this observer, these bacilli may be retained in the lung tissue for months at a time, remaining latent awhile, and then becoming active again, with a resulting exacerbation of the disease. Consumptives are particularly susceptible to attacks of influenza. Williams, in the examination of washed sputa in cases of pulmonary tuberculosis, has on numerous occasions found abundant influenza bacilli, and this in the summer, when no influenza was known to be present in New York. Taken together with Pfeiffer's results in Berlin, this indicates that at all times of the year many consumptives carry about with them influenza bacilli, and that very likely many healthy persons also harbor a few. Given proper climatic conditions, we have at all times the seed to start an epidemic.

The influenza bacillus does not occur, as a rule, in the blood. According to Pfuhl and Nauwerck, the influenza bacilli have been found in the interior organs and the brain, but these observations require further confirmation. So far as positive results have shown, influenza would seem to be a local infection confined to

the air-passages; the general symptoms produced are due probably to the absorption of the toxic products of the specific organism, these poisons being particularly active in their effects on the central nervous system.

The discovery of this bacillus enables us to explain many things, previously unaccountable, in the cause of epidemic influenza. We now know, from the property of the influenza bacillus not being able 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 the fact that the bacilli, as already mentioned, often remain latent in the lungs or bronchial secretions of the body for many months, and perhaps years, and then become active again, when under favorable circumstances they may be communicated to others. The bacteriological diagnosis of influenza is of considerable importance for the identification of clinically doubtful cases, which, from their clinical symptoms, may be mistaken for grippe, or *vice versa*, such as bronchitis, pneumonia, or tuberculosis. Up to the present time, however, the diagnosis gives us little help in prognosis or treatment.

In acute uncomplicated cases the probable diagnosis can be frequently made by microscopical examinations of stained preparations of the sputum, there being present enormous numbers of small bacilli. In chronic cases or those of mixed infection the culture method usually gives a positive result. The bacillus of influenza is so

well characterized by its morphological, staining, and cultural peculiarities that it may be distinguished with sufficient certainty for practical purposes from all other bacteria by an expert bacteriologist who is familiar with it. The only bacillus which resembles it at all closely is the pseudo-influenza bacillus found by Pfeiffer in three cases of bronchopneumonia. This bacillus is culturally very similar to the true bacillus *influenzæ*, 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. There is no doubt that other infections are also included under the clinical forms of influenza, and during an epidemic 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 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 diagnosticated 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 and amount of the poisons than to the special variety of organisms producing them.

CHAPTER XXI.

DIPHTHERIA BACILLUS.

History. The specific contagious disease which we now call diphtheria, and, therefore, according to our present belief, the bacilli which cause it, can be traced back to almost the Homeric period of Grecian history. The Greeks believed that it had been communicated to their country from Egypt. The description of the pharyngeal and laryngeal manifestations of this disease left by Aretæus leaves no doubt that it was of diphtheria that he wrote. Galen, in his remarks on the Chironion ulcer, tells us that the pseudomembrane was gotten rid of by coughing in the laryngeal form of the disease, and by hawking in the pharyngeal type. From time to time during the next one thousand years we hear of epidemics both in Italy and in other portions of the civilized world which indicate that the specific bacteria continued to be handed down from case to case. In 1517 we read of a malignant form of the disease raging in Switzerland, along the Rhine, and in the Netherlands. In 1557 we read of further epidemics in France, Germany, Holland, and Spain. The disease now crossed to America, and in the New England States we get clear accounts of its ravages. Thus, Samuel Danforth, in 1659, lost four of his eleven children within a fortnight by a "malady of the bladders in the windpipe." In 1765, Home, a Scotchman,

tried to show that "croup" and pharyngeal diphtheria were different diseases, or, in bacteriological terms, due to different micro-organisms, and this subject remained under controversy until it was recently settled that while most cases were undoubtedly due, at least to a great extent, to diphtheria bacilli, a few were not.

Bard, an American, supported, in 1771, the opposite theory from Home, considering the process the same wherever located. In this ground he was much nearer to the facts than Home. His observations upon diphtheria were very important and accurate.

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 micro-organisms to the disease began to be recognized. Since then the combined clinical, bacteriological, and pathological studies have sufficed to make diphtheria one of the best understood of diseases.

The Bacillus. In the year 1883 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. One year later Löffler published the results of a very thorough and extensive series of investigations on this subject. He found the bacillus described by Klebs in many cases of throat inflammations which had been diagnosticated as diphtheria. He separated these bacilli from the other bacteria present and obtained them in pure culture. When he inoculated the bacilli upon the abraded mucous membrane of susceptible animals, more or less characteristic

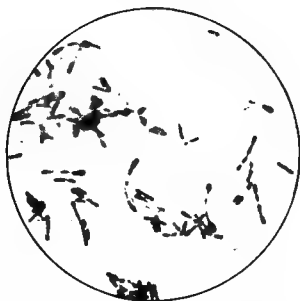
pseudomembranes were produced, and frequently death or paralysis followed, with characteristic lesions.

In 1887 and 1888 further studies by Löffler, Roux, and Yersin added to the proof of the dependence of diphtheria on this bacillus. It was found that while no other forms of bacteria were constantly met with, the diphtheria bacilli were present in all characteristic cases of diphtheria, and that these bacilli possessed the morphological, cultural, and pathogenic qualities of those described by Klebs and Löffler. The results of these investigations have since been confirmed by a great number of combined clinical and bacteriological observations both in animals and human beings. A very instructive accidental experiment was carried out under my observation some years ago. One of the laboratory workers unintentionally sucked through a defective pipette a few drops into the mouth of a bouillon culture of a virulent diphtheria bacillus, and two days later characteristic diphtheria of a serious type developed. All the conditions have been fulfilled for diphtheria which are necessary to the most rigid proof of the dependence of an infective disease upon a given micro-organism—viz., the constant presence of this organism in the lesions of the disease, the isolation of the organism in pure culture, the reproduction of the essential lesions of the disease in animals and in man by inoculation with pure cultures, the failure to produce all the characteristic lesions of this disease by any other bacteria, and the additional proof of the immunizing value of the specific substances developed in animals subjected to injections of diphtheria toxin. In view of these facts we are now justified in saying that the name diphtheria, or at least primary diphtheria, should be

applied, and exclusively applied, to that acute infectious disease usually associated with pseudomembranous affection of the mucous membranes which is primarily caused by the bacillus diphtheriæ of Löffler. Other bacteria do, indeed, occasionally produce lesions which simulate in one way or another those caused by the diphtheria bacillus, but none of them ever produce lesions similar in their totality to those of a characteristic case of diphtheria.

Morphology. When cover-glass preparations made 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 0.2μ to 0.8μ and the length usually

FIG. 41.



One of very characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain. $\times 1100$ diameters
Stain, methylene-blue.

FIG. 42.



Extremely long form of diphtheria bacillus. This culture has grown on artificial media for four years and produces strong toxin. $\times 1100$ diameters.

from 1μ to 6μ , but exceptionally even longer (see Fig. 42). They occur singly and in pairs (see Figs. 41 to 44), and very infrequently in chains of three or four. At times, especially in the tissues, branching forms are

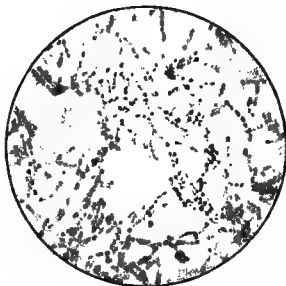
observed. The rods are straight or slightly curved, and usually are not uniformly cylindrical throughout their entire length, but are swollen at the end or pointed at

FIG. 43.



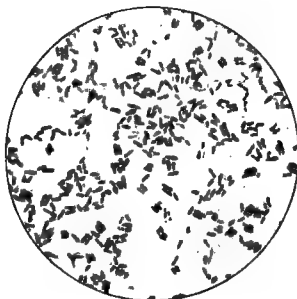
Diphtheria bacilli characteristic in shapes but showing even staining. In appearance similar to the xerosis bacillus. $\times 1100$ diameters. Stain, methylene-blue.

FIG. 44.



Non-virulent diphtheria bacilli, showing stain with Neisser's solutions, supposed to be characteristic of virulent bacilli. Bodies of bacilli in smear, faint brown; points, dark blue.

FIG. 45.



Small type of pseudodiphtheria bacilli. $\times 1000$ diameters.

the ends and swollen in the middle portion. The average length of the bacilli in cultures from different sources frequently varies greatly, and even from the

same culture individual bacilli differ much in their size and shape. The two bacilli of a pair may lie with their long diameter in the same axis or at an obtuse or an acute angle, or the pairs of bacilli may lie side by side or irregularly across each other. The bacilli possess no spores, but have in them highly refractile bodies at certain stages in their life.

The Klebs-Löffler bacilli *stain* readily with ordinary aniline dyes, and retain fairly well their color after staining by Gram's method. When Löffler's alkaline solution of methylene-blue is applied cold for five minutes or warm for one minute the bacilli from blood-serum cultures especially, and from other media less constantly, stain in an irregular and extremely characteristic way (see Fig. 41). Many of 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. 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. The bacilli seem to stain in this peculiar manner at a certain period of their growth, and more when grown on some media than on others, so that only a portion of the organisms taken from a culture at any one time will show the characteristic staining. In old cultures the bacilli stain poorly and not at all in a characteristic way. 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 to eighteen hours' growth on serum nearly all will show the blue bodies (Fig. 44), while with the pseudotype (Fig. 45), to be described hereafter, few, if any, 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 “

No. 2.

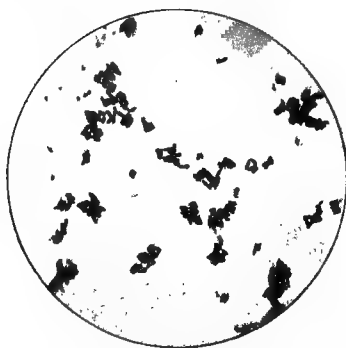
Bismark brown	1 part.
Boiling distilled water	500 parts.

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 any more information as to the virulence of the bacilli than the usual methylene-blue solution of Löffler. A small percentage of virulent bacilli fail to take the Neisser stain, and quite a few non-virulent pseudodiphtheria bacilli show the dark bodies. In New York there are also a large number of bacilli which seem to have all the staining and cultural characteristics of the virulent bacilli, and yet are non-virulent in the sense that they produce no specific

toxin. To one who is accustomed to the Löffler stain it gives as much information as any other as to the specific virulence of the bacilli. The Neisser stain will undoubtedly cause the examiner to suspect more strongly some bacilli of being virulent than the Löffler stain, but with the varieties met with in New York this suspicion is as apt to be wrong as right. As will be stated more fully later, nothing but the animal inoculations with control injections of antitoxin will separate specifically virulent from non-virulent bacilli.

The morphology of the diphtheria bacillus varies considerably with the different culture media employed. On glycerin agar or simple nutrient agar it is smaller, and, as a rule, more regular in form than when grown on other usual culture media (Fig. 46). Short, spindle,

FIG. 46.



Diphtheria bacilli from agar culture. $\times 1000$ diameters.

lancet, or club-shaped forms, staining uniformly, are here commonly observed. The bacilli which have developed in the pseudomembranes or exudate in cases of diphtheria resemble in shape those grown on blood-serum, but stain more evenly.

But though the morphology of the diphtheria bacillus is more regular under some circumstances than others, its chief morphological characteristic is its irregularity of form and size.

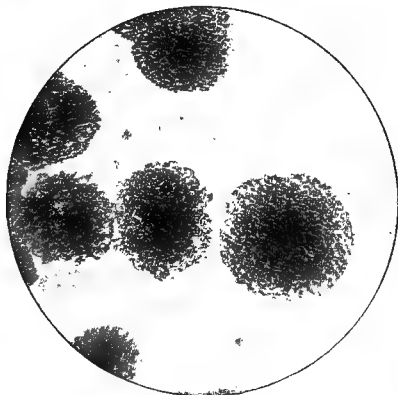
Biology. The Klebs-Löffler bacillus is non-motile and non-liquefying. It is *aërobic*. It grows most readily in the presence of oxygen, but also without it; it is thus *facultative anaërobic*. It does not form spores. Its thermal death-point with ten minutes' exposure is about 58° C., and with longer exposure a lower temperature; it is more easily destroyed by disinfectants than many other bacteria. In the dry state and exposed to diffuse light diphtheria bacilli usually die in a few days or may live for weeks or months; when in the dark, or protected by a film of mucus or albumin, they may live for even longer periods. Thus I 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; 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 sensitive to cold, for I found it to retain its virulence after exposure for two hours to several hundred degrees below zero. It begins to develop, but grows slowly, at a temperature of 20° C., or even less. It grows more rapidly as the temperature rises, and attains its maximum development at 37° C. It may grow at a temperature as high as 41° C. and retain its virulence for months.

Growth on Blood-serum. Blood-serum, especially in the form of Löffler'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. 377. If we examine the growth of the 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 the blood-serum.

Growth on Agar. On 1 per cent. slightly alkaline, plain 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. 30, page 229, and Fig. 47, page 339.) The diphtheria bacillus obtained from cultures which have developed for some time on culture media grows well, as a rule,

on suitable nutrient or glycerin-agar, but when fresh from pseudomembranes it frequently grows on these media with great difficulty, and the colonies develop so slowly as to be covered up by the more luxuriant growth of other bacteria, or fail to develop at all.

FIG. 47.

Colonies of diphtheria bacilli. $\times 200$ diameters.

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 very much in their thickness. Some are almost translucent; 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. With a high-power lens the edges show sprouting bacilli. The colonies are gray or grayish-white by reflected light and pure gray with an olive tint by transmitted light.

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 few bacilli, no growth whatever may occur, while the tubes of coagulated blood-serum inoculated with the same exudate contain the bacilli abundantly. Again, agar prepared from broth made from different specimens of beef or to which different peptones have been added, varies as to its suitability for the growth of the bacilli. Because of the uncertainty, therefore, of obtaining a growth by the inoculation of agar with bacilli unaccustomed to this medium, agar is a far less reliable medium than blood-serum for use in primary cultures for diagnostic purposes. If used the agar should at least be tested by means of a culture before being employed. A mixture composed of two parts of a 1½ 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. The mixture is made by adding the warmed ascitic fluid to the tubes containing the melted agar cooled to 60°. After shaking the Petri plates are filled.

The Isolation of the Diphtheria Bacillus from Plate Cultures. Nutrient plain or glycerin-agar, with or without the addition of ascitic fluid, is, however, the

medium employed to get by plating methods a pure culture from the original serum tube. The agar should be freshly melted and poured in the Petri dish for this purpose. After it has hardened the layers in a number of plates are 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 the bacteria, selected, as a rule, from the drier portion of the serum. The plates are left in the incubator for twelve hours at 37° C. In the examination of the plates one should first seek for typical colonies and then later for any that look nearest the characteristic picture. Diphtheria colonies are very apt to be found at the edges of the streaks of bacterial growth.

Growth in Bouillon. The diphtheria bacillus usually grows readily in broth slightly alkaline to litmus. 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 alkaline 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 more 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 pedicle. The diphtheria bacillus in its growth causes a fermentation of the meat sugars and the glucose, and thus changes the reaction of the bouillon, rendering it distinctly less alkaline within forty-eight hours, and then, after a vari-

able 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 Bouillon. Many diphtheria bacilli grow but feebly in nutrient bouillon when first removed from the throat. These develop more luxuriantly when to the bouillon 25 per cent. ascitic fluid or blood-serum is added.

Growth on Gelatin. The growth on this medium is much slower, more scanty, and less characteristic than that on the other media mentioned, on account of the lower temperature at which it is used.

Growth in Milk. The diphtheria bacillus grows readily in milk, beginning to develop at a comparatively low temperature (20° C.). Thus milk having become inoculated with the bacillus from some cases of diphtheria may under certain conditions be the means of conveying infection to previously healthy persons. Though this growth takes place, the milk remains unchanged in appearance.

Pathogenesis. The diphtheria bacillus is 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 virulence of diphtheria bacilli from different sources, as measured by their toxin production, varies enormously. Thus 0.002 c.c. of a forty-hour bouillon

culture of one bacillus will kill a guinea-pig, while it would require 1 c.c. of the culture of another bacillus to kill. The same marked variation occurs in the amount of toxin produced by different bacilli in their growth in media outside of the body. There are also bacilli which produce no specific toxin whatever and yet appear to have all the other characteristics of virulent bacilli. Moreover, the diphtheria bacilli differ greatly in the tenacity with which they retain their virulence when grown outside the body. The bacillus that we have used in the laboratory of the health department has retained its virulence unaltered for four years in frequently renewed bouillon cultures. Other bacilli have lost 50 per cent. of their virulence after being kept for only a few months. The passage of diphtheria bacilli through the bodies of susceptible animals does not increase their virulence to any considerable extent, this being probably due to the fact that the bacilli multiply but little in the tissues.

At the autopsy of animals dying from the poisons produced by the bacilli the characteristic lesions described by Löffler 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 pleural and the pericardial, frequently contain an excess of fluid, usually clear, but at times turbid; the lungs are generally congested. In the organs are found numerous smaller and larger masses of necrotic cells, which are permeated by leucocytes. The heart and voluntary muscular fibres usually show degenerative changes. Occasionally there is fatty degeneration of the liver and

kidneys. The number of leucocytes 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 other portions of the body and causing death by paralysis of the heart or respiratory organs, is also produced in many cases in which the inoculated animals do not succumb to a too rapid intoxication. In rare instances the muscles of the neck or of the larynx are first paralyzed, and thus characteristic symptoms are caused. In a number of animals I have seen recovery take place three to six weeks after the onset of the paralysis.

Diphtheria Toxin. It is evident that a micro organism which, when injected subcutaneously, destroys the life of susceptible animals and produces such marked anatomical changes in the internal organs, while it is found only at or near the point of inoculation, must owe its pathogenic power to the formation of a poison which, being absorbed, gives rise to toxæmia and death. This poison or *toxin* has been partially isolated by Roux and Yersin, and others, 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 (see page 72). 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 from light and air and in cold storage it keeps almost unaltered for years.

The Production of Toxin in Culture Media. The artificial production of toxin in 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 theoretical 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; or it may be that some conditions favor some bacilli while they are deleterious to others. Diphtheria bacilli may find conditions suitable for luxuriant growth, but unsuitable for the production of toxin. The requisite conditions for a good development of toxin, as judged by the behavior of a number of cultures, are a temperature from about 35° to 37.5° C., a suitable culture medium, such as a 2 per cent. peptone nutrient bouillon of an alkalinity which should be about 8 c.c. of normal soda solution per litre above the neutral point to litmus, and prepared from a suitable peptone 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, 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. In neutral bouillon, as pointed out by Smith and Spronck, the sugar does produce sufficient acid to interfere with the growth of the bacilli and the development of toxin. This can be prevented by the previous destruction of the sugar through the fermentation caused by the growth of the colon bacilli. After the fermentation 0.1 per cent. of glucose should be added. Beside the sugar and allied bodies in the meat there are other substances, whose nature is unknown, which hinder or aid a full growth of the bacilli or production of toxin. This is true of bouillon made directly from fresh meat, fermented meat, or meat extracts. 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 fermented bouillon. Contradictory results have been obtained by others, and must be attributed to the difference in the materials used.

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, 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 seems to aid in the development of toxin.

The relations of toxin to antitoxin will be described after the subject of antitoxin has been considered.

Non-virulent Diphtheria Bacilli. Xerosis Bacilli. In the very large number of tests for virulence of the bacilli obtained from hundreds of cases of suspected diphtheria which have been carried out during the past six 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 Löffler 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-Löffler bacillus, yet producers, at least in artificial culture media and

the usual test animals, of no appreciable diphtheria toxin. Between bacilli which produce a great deal of toxin and those which apparently produce none we find all grades of virulence. We believe, therefore, that in accordance with Roux and Yersin these bacilli should be considered as attenuated varieties of the diphtheria bacillus which have lost their power to produce diphtheria toxin. These observers, and others following them, have shown that the virulent bacilli can be artificially attenuated by cultivating them at a temperature of 39.5° to 40° C. in a current of air. So far as we know, bacilli which produce no specific toxin have never 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 lose it more quickly. 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 conjunctivæ by Kuschbert and Neisser, 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. Fig. 43, though taken from virulent bacilli, gives an exact picture of many of the xerosis variety. These bacilli may be almost non-pathogenic in guinea-pigs, or they may kill, as we have found in a number of instances, in doses of 2 to 5 c.c. hypodermatically 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. In this very same location, however, diphtheria bacilli are found of very low toxic power, so that here, again, we cannot assert that these xerotic bacilli have not come from true diphtheria stock.

Location of Diphtheritic Inflammations and Virulence of Bacilli. Virulent bacilli produce and are found not only in pseudomembranous inflammations of the fauces, larynx, and nasal cavities, but also occasionally in membranous affections of the skin, vagina, rectum, conjunctiva, nose, and ear (simple membranous rhinitis and otitis media). From the severity of an isolated case the virulence of the bacilli cannot be accurately determined. The most virulent bacillus I have ever found was obtained from a mild case of diphtheria simulating tonsillitis. Another case, however, infected by this 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.

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 inherited or acquired. 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, poor 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) I 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 non-virulent bacilli or attenuated forms of the diphtheria bacillus were found. Very similar observations have been made by others in many widely separated countries.

The 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, and after the disappearance of the exudate, has been repeatedly demonstrated. Beebe and I found that in 304 of 605 consecutive cases the bacilli disappeared within three days after the disappearance of the pseudo-membrane; 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 I have met with a case in which they persisted for six months.

Pseudodiphtheria Bacilli. Beside 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 Löffler 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 Löffler bacillus (Fig. 45). On blood-serum their colony growth is very similar to that of the diphtheria bacilli. The great majority of them in any 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 about 1 per cent. of the normal throat and nasal secretions, in New York City, and seem to have now at least no connection with diphtheria; whether they were originally derived from diphtheria bacillus is doubtful; they certainly seem to have no connection with it now. They never produce diphtheria toxin, and to them properly has been applied the name *pseudodiphtheria bacilli*. In bouillon they grow, as a rule, less luxuriantly than the diphtheria bacilli. 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. The Neisser staining method is of value here, but, unfortunately, the absence of the stained bodies is not a sufficient ground to exclude the possibility of their being true diphtheria bacilli. There are also some varieties which resemble the short pseudo-bacilli 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.

Mixed Infection in Diphtheria. Virulent diphtheria bacilli, however, are not the only bacteria present in human diphtheria. Various cocci, more particularly streptococci, staphylococci, and pneumococci, are almost always found associated with Löffler bacilli in diphtheria, playing an important part in the disease and leading often to serious complications (sepsis and bronchopneumonia). Indeed, the prognosis in a case of diphtheria is now judged to be graver, other things being equal, according to the degree in which other pathogenic bacteria influence the course of the disease. These cases of so-called *mixed infection* in diphtheria have within recent years attracted considerable attention, and have been the subject of a number of animal experiments. 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 Löffler bacillus into the deeper tissues or supplying needed conditions for the development of its toxin. Thus diphtheria is not always a primary, but often a secondary disease, following some other infection, as measles or scarlet fever. 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 micro-organism 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 which, under suitable conditions, take an active part in producing this form of inflammation. Some of these bacteria do not develop on artificial media, so that we know little of their characteristics. Among these is a long slender bacillus which is occasionally found in great abundance in the middle layers of pseudomembranes when the diphtheria

bacillus is absent. This, or one similar to it, has been described by Vincent.¹ It does not grow on artificial media and is not pathogenic in animals. From its presence in the false membrane of a number of cases, it is believed to have some causal relation to them.

These cases show most of the local appearances of true diphtheria, the superficial necrosis of the epithelium, the membrane of the glandular swellings. The pseudomembranes may persist for from one to two weeks, or even, in exceptional cases, longer. This bacillus is apparently frequently present in the normal throat, and is probably only able under certain favoring conditions, such as syphilis, to produce lesions. Nerve degeneration and paralysis do not follow an attack.

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 covering the inflamed tissues in these non-specific cases is, it is true, usually but not always, rather an exudate than a true pseudomembrane. The majority of these cases, however, are mild affections, being only of importance in adding to the severity of the disease which they complicate. An exception should be made when the larynx is affected, as here the lungs are often secondarily involved. The bacteria which occur in *false diphtheria* are streptococci, staphylococci, diplococci, and sometimes pseudodiphtheria bacilli or bacilli which are morphologically and culturally distinct from the

¹ Annales de l'Institut Pasteur, August, 1899.

Löffler bacilli. These will be referred to further under their respective organisms.

The Transmission of Diphtheria. The possibility of the transmission of diphtheria from animals to man cannot be disputed, for cats and many 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 due to other micro-organisms than the diphtheria bacilli. Diphtheritic infection, however, can generally be traced, directly or indirectly, to its source; though there are undoubtedly some cases of diphtheria in which we cannot determine the source of the infection, for we have no reason to believe that diphtheria is ever spontaneous.

Let us consider some of the means by which the disease may be communicated. In actual experiment the bacilli have been observed to remain virulent in bits of dried membrane by Löffler for fourteen weeks, by us for seventeen weeks, and by Roux and Yersin 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, on drinking-cups, shoes, hair, slate-pencils, etc. Beside 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 conditions there suitable for their growth or are swept away by food or drink before they could effect a lodgement.

Susceptibility to and Immunity against Diphtheria. An individual susceptibility, both general and local, to diphtheria, as in all infectious diseases, is necessary to contract this disease. Moreover, the diphtheria poison does not produce the same effect on the mucous membranes of all persons. Age has long been recognized to be an important factor in diphtheria. Children within the first six months of life are but little susceptible, the greatest degree of susceptibility being between the third and the tenth year, while adults are almost immune. An inherited susceptibility or "family predisposition" to the disease has also been observed.

Long before the discovery of the Klebs-Löffler bacillus it was a well known fact that two attacks of diphtheria seldom occurred in the same individual within short periods of time, and none of us would fear to leave a convalescent case in the same room with one still suffering from the disease. To what this natural susceptibility or immunity is due is still only partially understood; when we remember, however, that simply a slight

increase in the acidity or alkalinity of the bouillon in which the diphtheria bacilli are producing their toxin will prevent further production, it is easy to imagine that many changes in the throat secretion or in its mucous membrane may prevent the development of the bacillus or of the production by the bacillus of its toxin, and, therefore, of its disease-producing power. But, as the result of animal experiments, it is now known that an artificial immunity against diphtheria can be produced, at least for a considerable length of time, by the development of substances directly antidotal to the diphtheria toxin. By the inoculation of virulent or somewhat attenuated cultures or of diphtheria toxin, Fraenkel, Behring, Wernicke, Aronson, Roux, and since then many others, have succeeded in immunizing animals; but the most important and valuable results are those which have been obtained by Behring, in conjunction with others, who 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 weeks or months. Moreover, the blood-serum of many individuals, usually adults, who have never had diphtheria often has a slight general antitoxic property.

Antitoxic Serum. The knowledge derived from these remarkable investigations into the protective powers of the blood-serum of immunized animals has been employed with the most brilliant results for the prevention and early treatment of diphtheria in man. The discovery of the method of the production of antitoxic

serum or antitoxin in animals, and its practical application to the treatment and cure of diphtheria, has been shared by many experimenters, at first chiefly in Germany and France, and later in this country. Among those whose labors in this direction have rendered them most worthy of mention are Behring, Ehrlich, Boer, Kossel, and Aronson in Germany; Roux, Martin, and Chaillou in France.

Results of the Antitoxin Treatment of Diphtheria. Though the results of the antitoxin treatment of diphtheria belong properly to the province of serum-therapy rather than to bacteriology, in view of the great practical importance of the subject it may not be amiss to quote here 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 :

“ 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 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 :

The strongest diphtheria toxin possible should be obtained by taking a very virulent culture and growing it under the conditions described on page 345. The culture, after a week's growth, is removed, and having been tested for purity by microscopical and culture tests is rendered sterile by the addition of 10 per cent. of a 5 per cent. solution of carbolic acid. On the following day the sterile culture is filtered through ordinary sterile filter-paper 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 0.01 c.c., when injected hypodermatically, should kill a 250-gramme guinea-pig.

The horses used should be young, vigorous, of fair size, and absolutely healthy. Vicious habits, such as

kicking, etc., make no difference, of course, except to those who handle the animals. A number of such horses are severally injected with an amount of toxin sufficient to kill five thousand guinea-pigs of 250 grammes' weight (about 20 c.c. of strong toxin). 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. With the first three injections of toxin 10,000 units of antitoxin are given. If antitoxin is not mixed with the first doses of toxin only one-tenth of the doses advised is to be given. At intervals of from five to eight days increasing injections of pure toxin are made, until at the end of two months from ten to twenty times the original amount is given. 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. If moderate grades only are desired, all that yield 100 units may be retained. 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. Very few horses

ever give above 1000 units, and none so far has given as much as 2000 units per c.c. The very best horses 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.

In order to obtain the serum the blood is withdrawn from the jugular vein by means of a sharp-pointed canula, 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 and allowed to clot, the flasks, however, being placed in a slanting position before clotting has commenced. The serum is drawn off after four days by means of sterile glass and rubber tubing, and is stored in large flasks. From this, as needed, 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 of the serum. An antiseptic may be added to the serum as a preservative, but it is not necessary and probably inadvisable, 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 40 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 10 per cent. for each month. The antitoxin in old serum is just the same as in that freshly-bottled, only there is less of it.

The nature of diphtheria antitoxin has until recently been known almost wholly from its physiological properties. Recently experiments have seemed to show that it was either closely bound to the globulins or was itself a globulin. Mr. J. P. Atkinson, assistant chemist in the laboratory, has kindly permitted me to state the results of his investigations, which will soon appear in the *Journal of Experimental Medicine*. He found that antitoxic and normal horse-serum react similarly toward MgSO_4 , in that the globulin is precipitated completely from the other constituents of the serum. 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. When watery solutions of this globulin are saturated with NaCl a precipitate occurs. When the solution is heated a series of further precipitates take place, as follows: Cloudiness appears at 40° , 49° , 57° , and 67° C.; complete precipitate occurs at 45° , 54° , 62° , and 72° C. Each of these precipitates has antitoxic properties, and the total quantities contain all the original antitoxin except some 5 per cent., which is evidently destroyed by the higher temperatures required for the last two precipitates. After the last precipitate the solution is free of globulin and also of all antitoxic properties.

A further fact developed by Atkinson is that the globulins increase markedly in the serum of horses as

the antitoxin strength increases. It seems, therefore, from the above facts that diphtheria antitoxin has the characteristics of the globulins. Whether it is a union of diphtheria toxin and globulin, or an increase of certain globulin-like substances through the stimulation of the toxin, we have as yet no facts to tell us. Antitoxin is destroyed by prolonged moderate heat (60° C.) and by short exposure to higher temperatures (95° to 100° C.). It is much less sensitive than diphtheria toxin.

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. As already stated, there is a great difference of opinion as to whether antitoxin acts by direct chemical neutralization of the toxin or indirectly on the cells. The facts in favor of a direct action of antitoxins upon their corresponding toxins have recently been briefly summarized by Cobbett as follows :

1. Certain reactions have been observed to take place between these substances outside the animal body (venom, ricin, croton, tetanus toxin, diphtheria toxin, and their corresponding antitoxins).

2. Various attempts to separate the toxins and antitoxins from neutral mixtures have been failures. Partial successes have, at least in some instances, been shown to depend upon the fact that insufficient time for their complete union was allowed, separation being no longer possible if this were granted.

3. The accuracy of the titration of toxins and antitoxins to within 1 per cent. of error.

4. The fact that 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 it-elf accounting for the difference.

5. The fact that the potency of antitoxin is greatly increased if it is allowed to come in contact with the toxin outside the animal body; and is increased still further if allowed to remain for sufficient time in contact with the toxin at a suitable temperature.

On the other hand, the conclusions which Buchner and Roux drew from their experiments have been shown to have been based on a misconception, for they ignored the capacity of an animal to deal with a certain minimal quantity of poison, and, consequently, made no distinction between a physiologically neutral and a completely neutral mixture.

The facts now known, therefore, indicate rather strongly that the antitoxins of tetanus and diphtheria, of snake-poison, of ricin, etc., enter into direct chemical combination with their respective toxins—a combination which is, perhaps, not exactly comparable to that of an acid with an alkali; for, as we have seen, it is a much slower one, but one which possibly—as Ehrlich has suggested—more closely resembles the formation of a double salt. Some facts seem to indicate that the antitoxin has a stronger affinity for toxin than the toxin has for the cells. Many points, however, are still far from clear as to the manner in which both toxins and antitoxins act.

The Testing of Antitoxin. This power, possessed by a definite quantity of antitoxin to neutralize a certain amount of toxin, is utilized in testing antitoxin. Guinea-pigs of about 250 grammes' weight are subcutaneously injected with one hundred or with ten fatal

doses of toxin which have been previously mixed with an amount of antitoxin believed to be sufficient to protect from the toxin. If the guinea-pig lives four days, but dies soon after, the amount of antitoxin added to the toxin was just 1 or 0.1 unit, according as one hundred or ten fatal doses were employed. If the guinea-pig dies earlier, less than 1 unit was added.

The Use of Antitoxin in Treatment and Immunization. The antitoxin in the higher grades is identical with that in the lower grades ; there is simply more of it in each drop of the serum. In treatment, however, for the same amount of antitoxin we have to inject less blood-serum with the higher grades, and, therefore, have somewhat less danger of rashes and other deleterious results. With concentrated globulin solutions we may hope still further to avoid all disagreeable effects (see page 362). The amount of antitoxin required for immunization is 200 units for an infant, 500 for an adult, and proportionately for those between these extremes. 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 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 units, and severe cases 3000 units. Where no improvement follows in twelve hours the dose should be repeated. Antitoxin is useless when given by the mouth, as very little of it is absorbed.

No deleterious effects are to be feared except a rash, with some rise of temperature, in about 20 per cent. of

the cases. With the serum from some horses the rashes are very infrequent, while with that from others they occur more often. The same horse will at one time furnish a serum which produces no rashes and at another one which gives a great number. No way has yet been found to eliminate them entirely. Filtering and moderate heating produce little effect. Standing for some months causes a precipitate to occur, and the clear serum seems somewhat less liable to produce rashes than when it was fresh.

The 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 three to five months all appreciable antitoxin has been eliminated. Immunity in human beings lasts from two to six weeks after an injection of 500 units of antitoxin.

Technical Points upon the Testing of Diphtheria Antitoxin and the Relations between the Toxicity and Neutralizing Value of Diphtheria Toxin. Until within a fairly recent time 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 Behring-Ehrlich definition of an antitoxin unit.

The results of tests by different experimenters with

the same antitoxic serum, but with different diphtheria toxins, proved this opinion to be incorrect. Ehrlich¹ 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 six 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 dys. when mixed with 1 anti-toxin unit "L ₊ " Ehrlich.	Fatal doses required to "completely neutralize 1 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.
2	0.03	42	32	10	Preserved two years.
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. four and eight days.
10	0.001	29.2	27.5	1.7	Precipitated from greatly deteriorated "toxin."
12	0.0025	100	50	50	Tested immediately after its withdrawal.

From the facts set forth in the tables, Ehrlich has derived interesting theories, which, if true, would add greatly to our knowledge of toxins, and would also have a very direct influence upon the present methods of standardizing antitoxin. He believes that the diphtheria bacilli in their growth produce a toxin which, so long as

¹ "Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen," Klinisches Jahrbuch, 1897.

it remains chemically unaltered, has a definite poisonous strength with a definite value in neutralizing antitoxin. This neutralization he believes to be a chemical union, in which two hundred fatal doses of toxin for a 250 grammes' weight guinea-pig combine with one unit of 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 full power to neutralize antitoxin. These substances, according to Ehrlich, fall into three groups. The first has more affinity for combining with the antitoxin than the toxin itself (protoxoids). The second has the same affinity (syntoxoids). The third has less affinity (epitoxoids).

According to him, if a mixture of toxoids and toxin is added to antitoxin, the protoxoids first combine with the antitoxin, then the syntoxoids and the toxin combine in equal proportions, so long as the supply lasts, with the amount of antitoxin remaining, or, if there is a surplus, with enough to satisfy them; finally, if any antitoxin remains, the epitoxoids unite with it.

If to a mixture in which all three toxoids, as well as toxin, have united with antitoxin, some additional toxic culture bouillon be added, the new protoxoids displace first the epitoxoids, and then, if free protoxoids remain, the toxin and the syntoxoids from their antitoxin, and thus liberate as well as add free toxin to the solution.

Ehrlich gives an interesting theory to explain the production of antitoxin in the blood. This he does upon the supposition that, when absorbed, the toxin combines with a portion of certain selected cells, and that this portion, by its union with toxin, becomes—at least physiologically—dead. The cell replaces this dead

matter with new and similar substance; after the stimuli following several repeated losses and replacements of this substance the cells produce it in excess. This substance, whether originally in the normal cell or reproduced there, and whether remaining in the cell or thrown out into the circulation, is antitoxin.

The above summary merely gives an outline of some of the points in Ehrlich's most interesting article. To become fully acquainted with the reason for his theories the article itself must be carefully read.

Interest in both his theoretical reasoning and in his practical conclusions led us to subject both to a series of tests which have, I believe, added some interesting facts to those already published by Ehrlich as well as cast doubts on some of his conclusions.

The results of these experiments of Atkinson and myself¹ 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 pro-

¹ Journal of Experimental Medicine, vol. iii., No. 4.

duction 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 150.¹ When we seek to analyze the above-described process we find certain facts which seem partly to explain it. Experiments have shown that filtered toxin, preserved for any length of time in conditions under which access of air occurs, gradually loses in both its toxicity and neutralizing power, and that it loses more rapidly in the former property than in the latter. Thus, while the fatal dose of a toxin preserved for one year rose from 0.01 c.c. to 0.55 c.c., it lost only half as much in neutralizing value, 1 unit neutralizing at first 1 c.c., at the end of the year 0.25 c.c. These processes take place more rapidly at room-temperature than in the ice-chest, and in the incubator than in the room.

In the fluid holding the living bacilli we have, therefore, 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 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

¹ L_+ = fatal doses of toxin required to kill a guinea-pig in four days after having been mixed with one unit of antitoxin.

L_0 = fatal doses of toxin required to fully neutralize one unit of antitoxin.

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.

Ehrlich's theories as to the splitting up of "toxin" into toxoids having little or no toxicity, but on the average full neutralizing power for antitoxin have not, in our opinion, been substantiated by the results of these experiments. The difference between the amount of toxin mixed with a unit of antitoxin which causes the first symptoms and that causing death upon the fourth day would be, it is true, explained by his theory; but the failure of this difference to be greater where, by his theories, epitoxoids should be in great abundance, prevents our acceptance of his views. The fact of the greater neutralization value of a fatal dose of a deteriorated toxin would be accounted for on his protoxoid theory. This, however, is not proof of its correctness, as other theories, such as the production by the diphtheria bacillus of two or more closely allied toxins, similar to the allied alkaloids produced by plants, would equally account for it if we supposed the one which had the greater neutralization value was more resistant to destruction than the other.¹ We only advance this theory to call attention to the fact that many theories can on paper explain a process without necessarily being thereby established.

While we do not believe, therefore, that he has changed the principles of testing antitoxin, yet we believe he has contributed greatly to uniformity in results by calling attention to the necessity of selecting

¹ The incomplete precipitation of the diphtheria toxin by $MgSO_4$ makes it probable that more than one poison exists.

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.

In spite of the great variations in the neutralizing value of a fatal dose in different toxins we do not believe there has been any such great difference in the toxins used by the different stations for testing purposes. Most laboratories have taken the culture fluid at about the time of its greatest toxicity, and the neutralizing value of a fatal dose of this toxin would seldom vary more than 10 per cent. above or below the standard now adopted in Germany by the government testing station, this latter being presumably as close as possible to that used to establish the original Behring-Ehrlich unit.

Where error has been made it has usually been by taking too old culture fluids, which would cause the antitoxin strength of samples tested to be estimated below and not above its real value. Culture 8, which is used not only by the New York Board of Health Laboratory but by many other laboratories in the United States and Europe, fortunately produces on the sixth to eighth day—the time at which the culture is usually removed—a toxin which grades Ehrlich's antitoxin within 5 per cent. of the strength given by him.

We believe that by using such a bacillus we can, after gaining a fuller knowledge of its characteristics, obtain a toxin of a known and suitable neutralizing value, and thus always correctly standardize an antitoxic serum. This is certainly true for the bacillus which we have used for the past four years. Meanwhile, a fairly permanent preparation of a carefully

tested antitoxin is of immense value in insuring a uniform, though not necessarily correct, standard among the different testing stations and in allowing of comparison between them.

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, must be modified so as to be defined as that amount of antitoxin which will neutralize one hundred fatal doses of a toxin similar to that adopted as the standard—namely, one having 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 100 times the amount of a toxin such as just described, which will kill 250 grammes of guinea-pig on an average in 96 hours. 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 of antitoxin for each c.c. When we mix only ten fatal doses of toxin with one-tenth of the amount of antitoxin used with 100 fatal doses we usually consider that the guinea-pig must not only live but remain well.

The Relation of Bacteriology to Diagnosis. I 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 inflamma-

tions of the throat belonging to both diphtheritic and to non-diphtheritic inflammations which appear exactly alike, having apparently similar symptoms and similar duration; that it is even possible to examine two cases, knowing that one is surely diphtheria, or at least that diphtheria bacilli are present in the exudate, and the other surely is not, and yet be unable to determine clinically which is true diphtheria and which is pseudo-diphtheria. 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. It is also true that now and then a case having this appearance is one of diphtheria, and almost every physician has seen such cases from time to time in households infected with diphtheria. 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 our doubt in our own 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. Cases in which no exudate is apparent and those in which the laryngeal obstruction is sudden and the spasmodic element is marked, are, however, frequently due to the activity of other bacteria. 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. Usually a small area of inflammation indicates a slight or moderate severity, and an extensive area a severe infection.

Most cases of pseudomembranes and exudates entirely

confined to portions of the tonsils in adults are not due to the diphtheria bacilli, although a few cases presenting these symptoms are. The more complete the involvement of the tonsils the more apt the case is to be due to them. 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. But from time to time such cases, if they have been exposed to diphtheria, may be complicated by it, and in some epidemics mixed infection is common.

The 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 the 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.

Bacteriological 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 the 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 easy 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 suitability of the media, 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. 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 BACTERIOLOGICAL DIAGNOSIS.
Collection of the Blood-serum and its Preparation for Use in Cultures. A covered glass jar which has been thor-

oroughly 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 Löffler blood-serum mixture. This is poured into tubes, which should be about four inches in length and two-thirds 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 2 c.c. are sufficient for each tube. 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

(Fig. 22) 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 corks and kept for months. The serum thus prepared is quite opaque and firm. A mixture of blood-cells renders the serum darker, but it is not less useful.

The Swab for Inoculating Culture Tubes. The swab 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, 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 a "culture outfit" has been devised, which consists 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.

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 microscopical 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 are 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 Löffler's solution of alkaline methylene-blue, and left without heating for 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 1/12 oil immersion lens—either an enormous number of characteristic Löffler-bacilli, with a moderate number of cocci, or a pure culture of cocci, mostly in pairs or short chains (see Streptococcus). In a few cases there will be an approximately even mixture of Löffler bacilli and cocci, and in others a great excess of cocci. Beside these, there will be occasionally met preparations in which, with the cocci, there are mingled bacilli more or less resembling the Löffler bacilli. These bacilli, which are usually of the pseudodiphtheria type of bacilli (see Fig. 46), 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 Microscopical Examination of the Exudate. An immediate diagnosis without the use of cultures is often possible from a microscopical 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 Löffler 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.

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. 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 for practical purposes as certainly 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 double the fatal dose of a broth culture of the bacilli to be tested; if the guinea-pig which received the antitoxin lives, while the control dies, it was surely a diphtheria bacil-

lus 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 the 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 gramme 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.

CHAPTER XXII.

THE BACILLUS OF TETANUS.

IN 1884, Nicolaier, a student in Flügge's Institute, produced tetanus in mice and rabbits by the subcutaneous inoculation of particles of garden earth, and showed that the disease was transmissible by inoculation from these animals to others. Carle and Rattone, in 1884, demonstrated the infectious nature of tetanus as it occurs in man. Finally, Kitasato, in 1889, obtained the bacillus of tetanus in pure culture and described his method of obtaining it and its biological characters.

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, 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.

Morphology. Motile, slender rods, with rounded ends, 0.3μ to 0.5μ 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. 48). It is *stained* with the ordinary aniline dyes, and is not decolorized by Gram's method. The spores may be demonstrated by double-staining with Ziehl's method.

FIG. 48.



Tetanus bacilli with spores in distended ends. $\times 1100$ diameters.

Biology. An *anaërobic, liquefying, motile* (though not very actively motile) bacillus. *Forms spores*, and in the spore stage it is not motile. It does not grow at temperatures below 14°C. , but grows slowly at temperatures from 20° to 24°C. , and best at 37°C. , when it rapidly forms spores. It will not grow in the presence of oxygen or carbon dioxide gas, but grows well in an atmosphere of pure hydrogen.

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 under an atmosphere of hydrogen.

Its growth in the animal organism is comparatively scanty, and is usually associated with other bacteria; hence, it is difficult to obtain it in pure culture. The method of procedure proposed by Kitasato, which, however, is not always successful, consists in inoculating an agar tube with the tetanus-bearing material (pus from the inoculation wound), keeping this for twenty-four to forty-eight hours at a temperature of 37° C., and, after the tetanus spores have formed, heating it for about an hour at 80° C., to destroy the associated bacteria. The spores of the tetanus bacillus being able to survive this exposure, anaërobic cultures are then made in the usual way, and the tetanus colonies thus isolated. The further development is unattended with difficulty. 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 the *bacillus subtilis*, and the resemblance to these colonies is soon lost. In old cultures the entire mass is made up of a number of fine threads, and the colonies are not unlike those of the common mould.

The colonies on *agar* are quite characteristic (Sanfelice). To the naked eye they present the appearance of light, fleecy clouds; under the microscope, a tangle of fine threads. The extreme fineness of the threads enables them to be distinguished from the colonies of other anaërobies.

The *stab cultures in gelatin* exhibit the appearance of a cloudy, linear mass, with prolongations radiating into the gelatin from all sides. 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 empyreumatic odor. It also grows in *acid culture media*, but of itself produces no acid. It develops in *milk* without coagulating it, and starch is not hydrated by it in its growth (Sanfelice).

The spores of the tetanus bacillus are very resistant to outside influences; they retain their vitality for months and years in a desiccated condition, and are not destroyed in two and a half months when present in putrefying material (Turco). They withstand an exposure of one hour to 80° C., but are killed by an exposure of five minutes to 100° C. in the steam sterilizer. They resist the action of 5 per cent. carbolic acid for ten hours, but succumb when exposed to it for fifteen 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. When acted upon for three hours by bichloride of mercury (1 : 1000) they are killed, and in thirty minutes when 0.5 per cent. HCl is added to the solution. If the solution contains 1 : 1000 bichloride, with 5 per cent. carbolic and a 0.5 per cent. HCl, the spores are killed in ten minutes. Silver nitrate solutions destroy the spores in one minute in 1 per cent. solution and in five minutes in 1 : 1000 solution.

Pathogenesis. In mice, guinea-pigs, rabbits, rats, 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—only as much as remains cling-

ing to a platinum needle—of an old culture is often sufficient to kill very susceptible animals like mice and guinea-pigs. Other animals require a larger amount. Birds are but little susceptible, and fowls scarcely at all. It is a remarkable fact that an amount of toxin sufficient to kill a hen would suffice to kill 500 horses. 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 here or in the interior organs other than these. 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 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 in the living body, but only produce lesions through the absorption of the poison which they produce at the point of infection. These authors also found that pure cultures of tetanus, after the germs had sporulated and the toxins had been destroyed by heat, could be injected into animals without producing tetanus. Even one or two millions of spores, if deprived of the toxins, proved harmless to guinea-pigs, and from 15 to 30 c.c. of broth cultures were harmless to rabbits. But if a culture of non-pathogenic organisms was injected simultaneously with the spores, or if there was an effusion of blood at the point of injection, or if there was a previous bruising of the tissues, the animals surely died of tetanus. Even irritating foreign bodies were 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 animals died. From these experiments it seems that a mixed infection is necessary to the development of tetanus when the infection is produced by spores.

This fact is of the greatest importance in natural tetanus. Here the infection may be considered as probably invariably 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. 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 to again cause the disease by inoculating an animal with the same splinter. The bacilli of tetanus are apparently more numerous in certain localities than in others—for example, some parts of Long Island and New Jersey, which 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.

Man and almost all domestic animals are subject to tetanus. 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. By successive inoculation of this pus in susceptible animals the disease can often be reproduced for from four to five generations; but sometimes there is a break in the chain, which proves that in such cases the infection has been brought about less by the bacilli than by the toxin which was transmitted with them.

Not only traumatic tetanus, but also all the various forms of tetanus, are now conceded to be produced by the tetanus bacillus—puerperal tetanus, tetanus neonatorum, and idiopathic and rheumatic tetanus. In tetanus neonatorum and puerperal tetanus the infection is introduced through the navel and the inner surface of the uterus. It should be borne in mind, however, that when there is no external and visible wound there may be an internal one. Carbone and Perrero report a case of so-called rheumatic tetanus in which attenuated forms of tetanus bacilli were found in the bronchial secretions. These bacilli possessed the morphological and cultural peculiarities of the tetanus bacilli, but they did not produce toxin. Similar anaërobes have been found in meat-juices and in the soil. The bacilli found in the bronchial secretions, therefore, may have been tetanus bacilli which, owing to certain conditions, had lost their virulence, just as we know it to happen

in diphtheria. It may well be supposed that the mucous membranes of the bronchi, and other similar membranes, in a condition of catarrhal inflammation, may be more susceptible to tetanus infection than they normally are.

Tetanus Toxin. It is evident from the localization of the tetanus bacilli at the point of inoculation and their slight multiplication at this point that they owe their action to the production of a powerful toxin. While there are a few cases on record in which the bacilli have been found in the tissues of the animal body other than the point of infection, the fact remains that in the vast majority of cases the tetanus bacillus is localized. This toxin can be readily separated from cultures by filtration. One-hundredth of a milligramme of an eight-day filtered bouillon culture is sufficient, as a rule, to kill a mouse. From this filtrate, however, the active toxic substance has been obtained in a much more concentrated form. The purified and dried tetanus toxin prepared by Brieger and Cohn was surely fatal to a 15 gramme mouse in a dose of 0.00000005 gramme. Reckoning according to the body-weight of 75 kilogrammes, or 175 pounds, it would require but 0.00023 gramme, or 0.23 milligramme of this toxin, to prove fatal to a man. By comparing this with snake-poison, Calmette has found that dried cobra venom requires 0.25 milligramme to kill a rabbit of 4 kilogrammes' weight, and according to body-weight, it would require 4.375 milligrammes to kill a man of 70 kilogrammes. As the fatal dose of atropine for an adult is 130 milligrammes, of strychnine from 30 to 100 milligrammes, and of anhydrous prussic acid 54 milligrammes, the appalling strength of the tetanus toxin

can readily be appreciated (Lambert). What the true composition and constitution of the tetanus poisons are is unknown. It has been shown, however, that it possesses neither the characteristics of an alkaloid (ptomain) nor of an albuminous body (toxalbumin); it is largely precipitated from fluids saturated with ammonium sulphate.

The quantity of the toxin produced varies, even when derived from one and the same culture, according to the age of the culture, its composition, reaction, etc.; and partly it is due to the extreme sensitiveness of the toxin, which cannot bear keeping any length of time or exposure to light, being 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 in the dry state.

Some authors (Kitasato and Sanfelice) have maintained that the tetanus cultures retain their virulence unaltered; others, again, have observed considerable alteration in toxicity. Righi, for instance, has observed that the tetanus bacillus cultivated under aërobic conditions may entirely lose its virulence. Certain chemical agents also produce on cultures of the tetanus bacillus an attenuation of virulence, if only a temporary one.

The Action of Tetanus Toxin in the Body. 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. According to Gumprecht, the action of tetanus depends upon an increased reflex excitability, as in strychnine-poisoning; but it is different from strychnine in its mode of distribution, and probably takes place chiefly through the nervous system, as in rabies. This view is supported by Brunner, Bruschetti, and others. Beck has described a peculiar degeneration in the motor cells of the cord in animals killed by tetanus. This degeneration does not seem to attack the entire cells, but only a peripheral part, and seems to be confined chiefly to the body of the cell, usually leaving the nucleus intact. Only very late do the nucleus and the nucleolus take part in the changes. The changes consist in a swelling of the cell and a homogeneous or finely granular degeneration with a swelling, and, finally, coarse lumping together of the chromatin. This is especially evident at the tiny eminence from which the axis-cylinder arises and in the axis-cylinder itself. Beck considers this as proving that the poison travels along the axis-cylinder, and that, as the nucleus is the last portion affected, the change is not a necrosis but only a modification of cell function.

But there is also, in addition, undoubtedly a diffusion of the poison by means of the blood and lymph. 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. Kitasato also found the serous exudates of the pleural

and pericardial cavities as well as the blood of tetanic animals would cause tetanus when transferred to other animals. Kahlmeyer, Bruschettini, and others have obtained similar results. The toxin has also been demonstrated in the urine when large amounts have been inoculated.

Courmont and Doyon believe that the so-called toxin elaborated by the tetanus bacillus is not the true poison, but is a ferment which forms from the poison in the body at the expense of the organism, and is found in the blood, sometimes in the urine, and in especial abundance in tetanized muscles. The action of tetanus toxin is never suddenly produced, though when once formed its absorption is rapid, but always requires a certain period of incubation. These authors hold that the substances produced by the tetanus bacillus must undergo a chemical change in the body, because after it is formed in the tissues it can be extracted from them by boiling, and when injected into other animals causes immediate tetanic symptoms without any period of incubation. But other observers repeating these experiments have failed to confirm Courmont and Doyon's results, and appear to have proved their theory to be untenable.

Tetanus Antitoxin. Behring and Kitasato were the first to show the possibility of immunizing animals against tetanus infection. Here the question of immunity against infection does not consist in producing an increased power of resistance against the development of the infecting agent, as is the case in most infectious diseases, but similar to diphtheria, in bringing about an immunity to the effects of the tetanus toxin. The bacillus of tetanus, as we have seen, does not belong

to the septicæmic class of organisms which spreads through the body, and by their growth and increase produce their effects, but, on the contrary, remains localized at the original point of infection. It produces, however, in its growth a most powerful toxin. The treatment of tetanus is, therefore, directed against the production of toxin and its neutralization in the body. The methods originally proposed by Behring and by Roux for producing a curative serum consisted chiefly in weakening the tetanus toxin by means of chemical disinfectants (iodine trichloride, Gram's and Lugol's solutions), so that when inoculated into the test-animals they produced comparatively little reaction. At the present time we inject the pure unaltered toxin either alone in small doses or along with antitoxin. After the first dose of toxin the animals acquire a certain tolerance which enables them to stand a dose of a less attenuated toxin or of a greater amount of unchanged toxin. Thus by gradually increasing the doses or the strength of the toxin administered, the animals are finally able to bear injections of large quantities of the strongest toxin.

These 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 thus shown 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; and from this it was assumed that the same result could be produced in natural tetanus in man; but, unfortunately, 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 often only on the appearance of tetanic symptoms, when the poison has already diffused itself through the body.

Tetanus Antitoxin. 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. After ten or fifteen days the culture fluid is filtered through porcelain, and the germ-free filtrate is used for the inoculations. The horses receive half a c.c. as the initial dose of a toxin of which 1 c.c. kills 250,000 grammes of guinea-pig, and along with this a sufficient amount of antitoxin to neutralize it. In five days this dose is doubled, and then every five to seven days larger amounts are given. The dose is increased, as rapidly as the horses can stand it, until they support 700 to 800 c.c. or more at a single injection. After some months of this treatment the blood of the horse contains the antitoxin in sufficient amount for therapeutic use. When the animals' temperatures are normal and they have recovered from the dose of toxin last given, they are bled into sterile flasks and the serum collected.

Technique of Testing Antitoxin Serum for Value in Antitoxin. Tetanus antitoxin is tested exactly in the same manner as diphtheria antitoxin, except that the standard unit is different. The test toxin used in the German method is one of which 1 gramme destroys 150,000,000 grammes of mouse. This is dissolved in $33\frac{1}{3}$ c.c. of 10 per cent. NaCl solution. Ten times the amount of antitoxic serum which neutralizes 1 c.c. of

this dilution of the test toxin contains one unit of antitoxin. 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 one gramme of animal. If 0.001 c.c. protected a 10 gramme mouse the strength of that serum would be 1:10,000. Guinea-pigs are sometimes used in place of mice. Knorr's toxin 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.

The Persistence of Antitoxin in the Blood. Ransom has recently shown that the tetanus antitoxin is eliminated just about as rapidly from the blood of an animal when produced by toxin injections as when injected with antitoxin, so long as the serum was from an animal of the same race. When from a different race, it is much more quickly eliminated. From this we see a possible 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.

He 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-sixths, 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 the antitoxic milk.

Results of the Antitoxin Treatment in Tetanus. Tetanus is a comparatively rare disease both in man and animals, though in some localities it is more common than in others. In New York city there are usually fifteen to thirty cases following every fourth of July. Most of them are caused by infection through blank cartridge wounds. Recovery sometimes follows from the ordinary symptomatic treatment or without treatment at all, so that the statistics of cures of the disease by the injection of antitoxic serum must be very carefully sifted before they can be accepted as reliable. Lambert, however, who has recently 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. The new method of injecting from 3–15 c.c. of antitoxic serum into the lateral ventricles has not, in the writer's opinion, shown itself to be superior to the intravenous or subcutaneous

methods. Some speak well of it. No bad results have followed the injections when the serum was sterile and the operation was performed aseptically.

The Dosage of Tetanus Antitoxin. For immunization 10 c.c. of a serum of a strength of 1:1,000,000,000 will suffice unless the danger seems great, when the injection is repeated at the end of a week. For treatment, it is well to begin with 50 c.c., and then, according to the severity of the case, give from 20 to 50 c.c. each day until the symptoms abate. In the gravest cases no curative effect will be noticed from the serum.

Though these few cases are not sufficient to form a final judgment of any treatment, Lambert concludes that by means of the antitoxin treatment, combined with other rational methods, the prognosis, even in acute cases of tetanus, has been improved; but that it still remains exceedingly grave—so much so that the preventive inoculation of serum in all cases where dirt has been ground into serious contusions deserves a much more extensive consideration than has heretofore been given it. The striking results which have been obtained, particularly 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.

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 micro-organism known produces similar effects to the tetanus bacillus,

nor is any other neutralized by tetanus antitoxin. The other characteristics also of this bacillus are usually distinctive, though microscopical 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.

CHAPTER XXIII.

BACILLUS TYPHOSUS (EBERTH-GAFFKY'S BACILLUS OF
TYPHOID FEVER ; BACILLUS TYPHI ABDOMINALIS).

THIS organism was first observed by Eberth, and independently by Koch, in 1880, in the spleen and diseased organs of the intestine in typhoid cadavers, but was not obtained in pure culture and its principal biological cultures described until the researches of Gaffky, in 1884. 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 almost impossible to produce infection or in any way give rise to lesions corresponding to those occurring generally in man. It has been recently shown, however, that animals under certain conditions, when their power of resistance has been reduced, as by exposure to the influence of noxious gases, may be rendered susceptible to infection, with the production of more or less characteristic lesions. These results, together with the specific reactions of the blood-serum of typhoid patients, as first pointed out by Pfeiffer, Gruber, Widal, and others, and the constant presence of the bacillus typhosus in the intestines and in some of the organs of the typhoid cadavers, as shown by its frequent isolation from the spleen, blood, and excretions of the sick during life and its absence in healthy persons,

unless they are convalescent from typhoid infection, have demonstrated, on a scientific basis, that this bacillus is the chief etiological factor in the production of typhoid fever.

Morphological Characters. The typhoid bacilli are rods of about 1μ to 3μ in length by 0.5μ to 0.8μ in diameter, with rounded ends, often growing into long threads. They are usually longer and somewhat more slender in form than the bacilli coli communis under similar conditions. The typhoid bacilli vary, however, in shape when grown in different culture media. (See Figs. 49, 50, and Fig. 6, page 39.)

FIG. 49.



Typhoid bacilli from nutrient agar.
 $\times 1100$ diameters.

FIG. 50.

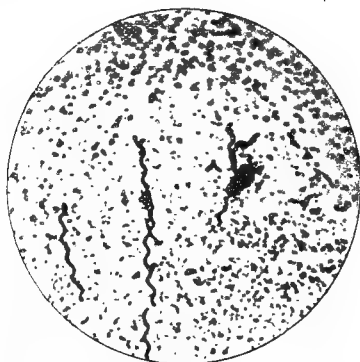


Typhoid bacilli from nutrient gelatin.
 $\times 1100$ diameters.

The typhoid bacilli *stain* with the ordinary aniline colors, but a little less readily than do most other bacteria, though there is no constant difference in staining characteristics between these and other bacilli of this group—the colon bacilli. They are decolorized by Gram's iodine solution. Not infrequently, particularly when grown on potato, refractive granules may

be seen at the ends of the rods, which stain more intensely, and either at the extremities or along the body "vacuoles" are observed, which remain unstained; but as these show even less resistant power than the homogeneous bacilli found in other cultures, they are certainly not spores, but probably are evidences only of retrograde changes and effects of the drying preparatory to staining.

FIG. 51.



Flagella, heavily stained, attached to bacilli.

The bacilli, when existing under favorable conditions, are, although in various cultures to a different degree, very actively motile, the smaller ones having often an undulating motion, while the larger rods dart about rapidly, with a snake-like movement. This movement is produced by a number of delicate locomotive organs in the form of fine, hair-like flagella, which are arranged around the bodies of the bacilli. (Fig. 10, page 43, and Fig. 51.) The flagella are usually from eighteen to twenty in number, but many short rods have but a single terminal flagellum. They are not seen in unstained preparations, nor are they rendered visible by

the ordinary methods of staining. (See Staining of Flagella, page 205.)

Biological Characters. The typhoid bacillus is a motile, aërobic, non-liquefying bacillus, developing best at 37° C.; over 40° and below 30° its growth is retarded; below 10° it ceases. It grows most abundantly in the presence of oxygen, but oxygen is not essential to its development.

Its growth on most culture media is similar to that of the bacillus coli communis, but it is somewhat slower and not quite so luxuriant.

FIG. 52.



A superficial and a deep colony of typhoid bacilli in gelatin.
× 50 diameters.

Growth on Gelatin Plates. (Fig. 52.) The colonies growing deep down in this plate medium have nothing in their appearance to distinguish them; they appear as round points with a sharp margin, of a yellowish-

brown color, and finely granular. 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.

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 streaked, granular, or uniform in structure, and of a yellowish-brown color. There is no liquefaction.

Growth in Bouillon. This medium is uniformly clouded by the typhoid bacillus, but the clouding is not so intense as by the colon bacillus. A film is frequently formed on the surface after eighteen to twenty-four hours' growth. A very slight amount of acid is produced.

Growth on Agar. The streak cultures on agar are not distinctive; a transparent, grayish streak is formed.

Growth on Potato. The growth on this medium has been held by some to be very important, but it varies considerably. When characteristic the growth is 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, not very luxuriant, and of the same color as the potato. 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 this

medium appear to be chiefly due to variations in the substance, especially in the reaction, of the potato.

Milk. The typhoid bacillus does not cause coagulation when grown in sterilized milk.

Fermentation. It does not produce fermentation in either glucose, lactose, saccharose, or glycerin bouillon, and evolves no gas as the result of fermentation.

Lactose-litmus Agar. It grows usually as pale blue colonies on lactose-litmus agar, but occasionally causes slight reddening of the surrounding medium.

Indol Reaction. It does not produce indol. 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 reaction, being a very delicate one, requires great care in its performance to arrive at accurate conclusions. (For test of indol, see page 77.) Instead of bouillon, the simple peptone-water (which consists of dried peptone, 1 part; sodium chloride, 0.5 part, and distilled water, 100 parts) is to be preferred for this purpose, because its pale color does not mask the reaction.

Pathogenic Properties. It has been extremely difficult to show experimentally that the bacillus typhosus is specifically pathogenic for animals. A great many experiments have been made, with the view of reproducing in the tissues of lower animals the pathological lesions of typhoid fever as seen in man, but the results have not been completely satisfactory; nor is this surprising when one considers that this disease does not occur naturally, so far as is known, among 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, not necessarily accompanied by the growth of the organism, rather than to infection due to the development of the typhoid bacillus in the tissues.

In a certain number of cases subcutaneous and intraperitoneal inoculations in animals have been productive of more or less typical typhoid lesions. Among the most successful efforts in this direction are the experiments of Cygnaeus and Seitz, who, by the inoculation of the typhoid bacillus 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, but their results were not constant. Of a number of experiments made by Abbott, with the same object in view, only one positive result followed the introduction of typhoid bacilli into the circulation of rabbits. In this case the ulcer in the ileum was macroscopically and microscopically identical with those found at autopsy in the small intestines of the human subject dead of this disease. The bacilli were found in the spleen.

Experiments indicate that the presence of other bacteria in the body, and of exposure to the effect of noxious gases in lowering the natural resistance of the individual, render him more susceptible to infection from typhoid fever and, indeed, from other infectious diseases.

But whatever conclusions may be drawn from these results, with regard to the typhoid process in animals, typhoid fever in the human subject is now recognized

as a true infection, caused by the introduction and growth of typhoid bacilli. It belongs to that class of infectious diseases which are known as metastatic—that is to say, diseases in which the specific bacilli do not abound in the entire circulation, as in septicæmia, nor remain localized in one place, but are distributed in groups throughout the body. The characteristic lesions of typhoid fever are seated in the lymphatic structures of the intestine—namely, the solitary follicles and patches of Peyer, the mesenteric glands, and the spleen. The liver and kidneys are less commonly attacked. Wherever found the typhoid bacilli are observed to be arranged in groups or foci; only occasionally, as in the walls of the intestine, are they singly or loosely aggregated together. These foci are formed, most probably, 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.

The production of the lymph-nodules so often found in typhoid fever in the internal organs is due to the effects of the toxic substances eliminated by the typhoid bacilli. This hyperplasia is particularly evident in the lymphatic structures of the intestine, these being more directly under the influence of the concentrated products of the bacilli. To these, however, other inflammatory processes are added, until finally necrosis or sloughing of the tissues takes place. Possibly all these series of changes may be at times caused solely by the products of the typhoid bacilli which are gathered at certain points. There is no question, however, that usually other organisms take part in the production of these processes in the intestines, but it remains to be determined when they begin to do so. In typhoid fever

necrosis of the tissues of the internal organs is of comparatively rare occurrence. Caseation of the mesenteric glands, which is commonly observed, is due probably to mixed infection. There are, however, a number of cases now on record in which the typhoid bacillus has played the part of *pus producer*. Cases of sacculated and general peritonitis, subphrenic abscess, osteomyelitis, periostitis, and inflammatory processes of other kinds have been reported as being due to the typhoid bacillus. Kruse also reports an abscess of the spleen which contained only bacillus typhosus, and typhoid abscess of the liver has been recorded by many. In certain cases of typhoid pneumonia, serous pleurisy, empyema, and meningitis, typhoid bacilli exclusively have occurred. The inflammation produced may or may not be accompanied by the formation of pus. As argument against the observations above cited there has been brought forward the supposition that probably the real cause of the disease had been destroyed before the entrance of the typhoid bacillus. Though this may be true of some cases, as in pneumonia, which is caused usually by the short-lived pneumococcus, there is no reason to doubt the causal relation of the typhoid bacillus to the other diseases, inasmuch as it has been proved by numerous investigations.

Such cases, however, are of comparatively rare occurrence, because only exceptionally do the bacilli sufficiently mass together in such numbers as to become pus producers. As a rule, when complications occur in typhoid fever they are due to secondary or *mixed infection* with the staphylococcus, pneumococcus, streptococcus, pyocyaneus, and colon bacillus. Frequently

these bacteria are found side by side with the typhoid bacilli; in such cases it is difficult to say which was the primary and which was the secondary infection.

The peculiar arrangement of the typhoid bacilli in the body can only be explained by their passage through the circulation; and this is proved by the bacilli being found in the spleen almost constantly and in smaller numbers in the blood itself. Thus, Neuhauss has had nine positive results out of fifteen in cultures from vein blood.

The typhoid bacillus can be transmitted also from the blood of the mother to the foetus (Eberth, Fraenkel, etc.). In one case reported by Ernst a living child, four days after birth, showed evidences of general typhoid infection, icterus and rose-spots. Frascani reports that in animal experiments he has frequently found typhoid bacilli in the foetus.

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 week of typhoid fever. Slight pathological lesions in the kidneys almost always occur in typhoid fever, but severe lesions also sometimes occur. In a case under our observation the urine was distinctly purulent and crowded with typhoid bacilli. The bacillus typhosus is not commonly found in the sweat, but Geisler observed it once. It has also been detected, though rarely, in the sputum and secretions of the throat.

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. According to Chiari, in typhoid fever the bacilli are almost always present in the gall-bladder. The bacilli

are frequently eliminated by the feces being derived from the inflamed mucous surface of the intestines; their growth within the intestinal canal itself, even if it occurs to a limited extent, is probably not extensive.

Methods of Infection. With regard to the *mode of invasion* of the typhoid bacilli, there is no doubt that it is principally by way of the mouth, through the stomach to the intestines. Mayer reports a particularly convincing illustration of this fact in a case where death ensued on the second day of the disease. On autopsy were found hyperæmia of the lungs, spleen, and kidney; in the lower portion of the ileum great enlargement of the solitary follicles and patches of Peyer, but nowhere a trace of necrosis or loss of substance; nor were the mesenteric glands enlarged. Microscopically an extraordinary deposit of characteristic bacilli were found in the submucosa and interstitial spaces of the muscles; many hundred bacilli lay in one field. On the other hand, several cases are recorded in which the intestinal changes were entirely wanting, and only a localization of bacilli and lesions in the mesenteric glands and spleen revealed the nature of the infection. Inasmuch as they were present in the lymph-glands which belong to the intestines, it may be assumed, thinks Kruse, who reports one of these cases, that the bacilli were here more rapidly absorbed than usual without multiplying to any extent in the intestines. The case mentioned by Guarnieri is also worthy of notice; in this there was apparently a primary infection of the gall-ducts, with no accompanying lesions in the intestine. Bacilli were found in the blood twelve days before death, and on autopsy pure cultures were obtained from the liver and spleen.

Not only do the very great majority of cases examined bacteriologically and pathologically, but the epidemiological history of the disease, prove 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 feces, the urine, and occasionally by the sputum.

Of considerable practical importance is it 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 very deficient. In feces the length of life of the typhoid bacilli is very variable; sometimes they live but a few hours, usually a few days, exceptionally for very long periods. Thus, according to Uffelmann, typhoid bacilli may remain alive in feces for five and a half months, and, according to Karlinski, for at least several months. Foote says that they can be found in living oysters for a month at a time. Their life in feces and in water, however, is usually very much shorter. As a rule, they can be detected in water no longer than fourteen days after introduction. 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 high temperature, light, desiccation, etc., to which it is peculiarly sensitive. That the bacilli do live much longer under favorable circumstances, as to protection and nourishment, than is generally supposed, is shown by the fact, as reported by Buschke, that they were found in an old bone-centre

seven years after the original infection. There is no reason to deny that such opportunities for a latent existence of the typhoid bacillus may not occur outside of the body. Indeed, many epidemics of typhoid fever can only be accounted for by some such assumption of latency in or outside of the body.

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 contamination of food by flies and other insects, or by the inhalation through the mouth of dust containing typhoid bacilli. Of the greatest importance, however, is the production of infection by contaminated drinking-water or through drinking-water or milk, which is the most plausible explanation for the majority of epidemics of typhoid fever. In many cases indirect proof of this mode of infection has been found in the known contamination of the water with typhoid feces or urine, and in some few cases it has been confirmed by direct proof in finding the bacilli. Examples of infection from water and milk have come frequently under our direct observation—for instance, a large force of workmen obtained their drinking-water from a well very near to their work. Typhoid fever broke out, and continued to spread until the well was filled up. Investigation showed that some of the sick, before their discovery, repeatedly infected the soil surrounding the well with their urine and feces. Another instance of milk infection secondary to water infection was the case of a milk dealer whose son came home suffering from typhoid fever. The intestinal movements were thrown into a small stream which ran into a pond from 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. In our late war, not only water infection but food infection was noticeable, 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.

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, overexertion, etc., as 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 is conducive 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. Specific *immunization* against experimental typhoid infection has been produced in mice, guinea-pigs, rabbits, dogs and other animals by the usual method of injecting at first small quantities of the living or dead typhoid culture and gradually increasing the dose. The blood-serum of animals thus

immunized has been found to acquire protective and curative bactericidal and perhaps 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 (Pfeiffer and Kolle, Widal and Chantemesse). Recently the attempt has been made to employ the typhoid-serum for the cure of typhoid fever in man, but no marked results have been obtained. The injection in man of very small amounts (0.3 c.c. of bouillon culture) of dead typhoid bacilli produces for a day or two a slight fever reaction, to be followed in a few days by the development of bactericidal substances in the blood, which apparently are sufficient in amount to give immunity for some weeks. The use of immunized serum, or when this cannot be obtained of dead cultures, would seem to be advisable where great danger of typhoid infection exists.

The Diagnosis of Typhoid Fever, or rather of Typhoid Infection, 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 in the way of diagnosis. In view of the interest which has been manifested in this test, and of the fact that it is now so largely used, a brief history of the investigations which led up to its discovery may be given.

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 grant 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 a portion of a fresh culture of the typhoid bacillus on agar is added to a small quantity of the serum of an animal immunized against typhoid 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, which were formerly and in control animals, which remain, actively motile and vigorous, become in a very short time, under the influence of the serum, entirely motionless and later dead. 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 takes place regularly 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 may serve for the differential diagnosis of the cholera vibron or typhoid bacillus from other vibrios or allied bacilli, such as Finkler's and Prior's or colon groups.

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 aid of 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 macroscopical 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, became and remained 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.

These 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*, so far as the cholera spirillum and typhoid bacillus, at least, are concerned. They conclude, nevertheless, that these investigations will render valuable assistance in the clinical diagnosis of cholera and typhoid fever. It developed through further research that before the development of the bactericidal substances agglutinative substances usually appeared in the blood.

WIDAL TEST. The first practical 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 fulness and detail in a communication published in June, 1896. Widal confirmed the reaction as above described, proved that the agglutinative reaction was one of infection and usually occurred early, elaborated the test, and proposed a method by which it may 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 it 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 employed in the New York Board 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.

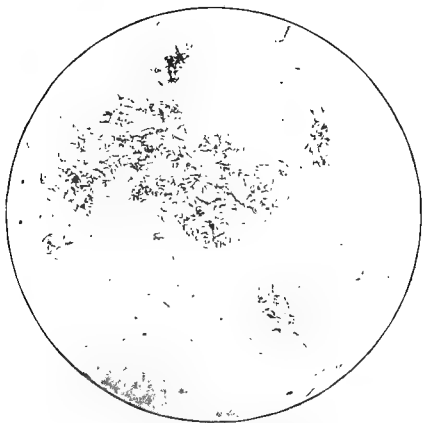
USE OF DRIED BLOOD. *Directions for Preparing Specimens of Blood.* The skin covering the tip of the finger or the ear is thoroughly cleansed, and is then pricked with a needle deeply enough to cause several drops of blood to exude. Two fair-sized drops are then placed on a glass slide, one near either end, and allowed to dry. 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 is brought into solution by adding to it and mixing it with about five times the quantity of water; 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, should have a faint reddish or pink tinge. The cover-glass with the mixture on the surface is inverted over a hollow slide (the edges about the concavity having been smeared with vaseline, so as to make a closed chamber), and the hanging drop then examined under the microscope (preferably by gaslight), a high-

power dry lens (about $1/8$ inch) being used, or, somewhat less serviceably, a $1/12$ oil-immersion lens.

THE REACTION. If the reaction takes place rapidly the first glance through the microscope reveals the completed reaction, all the bacilli being in loose clumps and nearly or altogether motionless (Fig. 53). Be-

FIG. 53.



Widal reaction. Bacilli gathered into one large and two small clumps, the few isolated bacteria being motionless or almost so.

tween 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 still less abundant the reaction may be watched through the whole course of its development. Immediately after mixing the blood and 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 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 proportions of blood added to the culture fluid.

Pseudo-reactions. If too concentrated a solution of dried blood from a healthy person is employed there will be an immobilization of the bacilli, but no true clumping. This is sometimes mistaken for a reaction. Again, 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 clumped 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.

USE OF SERUM. *Mode of Obtaining Serum for Examination.* Fluid blood-serum can be easily 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 wax, and as the blood clots a few drops of serum separate. This method of obtaining blood-serum has the advantage of rapidity; but it has also disadvantages—namely, that the serum thus separated is apt to contain more or less blood-cells, which somewhat obscure the field when the liquid serum is immediately mixed with the culture, and the needle stab is often objected to. Second, the serum may be obtained *from blisters*. This gives more satisfactory results, but causes twelve hours' 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 be easily obtained from a blister so small that it is practically painless and harmless. The serum obtained is clear and admirably suited for the test.

Advantages and Disadvantage of Serum and Dried 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 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, 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 six 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.

The Typhoid Culture Employed. It is important that the culture employed for serum-tests should be a suitable one, for although in our experience all cultures show the reaction, yet some respond much better than others. A broth culture of the typhoid bacillus developed at 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. Stock cultures of typhoid 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.

The Dilution of the Blood-serum to be Employed and the 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 the agglutinating and immobilizing substances produced in the blood of a patient suffering from typhoid infection are the same as those present at times in normal blood, or those produced in the blood of persons sick from other infections. It is intended, however, to maintain that the effect upon the bacilli, as seen under the microscope, is identical, the difference being that in typhoid fever, as a rule, substances which cause this reaction are usually far in excess of the amount 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.

The results obtained in the health department laboratories, as well as elsewhere, have shown that in a certain proportion of cases not typhoid fever there occurs a delayed moderate reaction in a 1 to 10 dilution of serum or blood (the proportion originally proposed by Widal); but very rarely, if ever, excepting in typhoid fever, or at least typhoid infection, does a complete reaction occur in this dilution within *five minutes*. When dried blood is used the slight tendency of non-typhoid blood in 1 to 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 by Fraenkel, Stern, Förster, Scholtz, ourselves and others, it has been found that in dilutions of 1 to 20 or more a decided, quick reaction is never produced in any febrile disease other than that due to typhoid infection, while in typhoid fever such a distinct reaction often occurs with dilutions of 1 to 50.

The mode of procedure, therefore, as now employed is as follows: The test is first made with the typhoid bacillus in a 10 per cent. solution of serum or blood. In the case of serum, one part of serum is added to nine of the bouillon culture. With dried blood, a solution of the blood is first made, and the final dilution guessed from the color of the mixed culture and blood solution. 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 considerable clumping

occurs—nothing more is needed; the result is negative as far as this specimen is concerned. If marked clumping and immobilization of the bacilli immediately begin and become complete within five minutes, this is denominated 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 to 20 and 1 to 50. If, however, upon examination of the mixture there is no marked immediate reaction, but the bacilli only show in the first few minutes an inhibition in their motility and a tendency to clump, which becomes more marked but not complete within five minutes, this must be tested with the higher dilution of 1 to 20, so as to measure the exact strength of the reaction. If in the 1 to 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 a probability only of typhoid infection has been established. The time allowed for the development of the reaction with the high dilutions is by many from one to two hours, but to us thirty minutes seem safer. Positive results obtained in this way may be taken to be conclusive unless there be grounds for suspecting that the reaction may be owing to a previous fairly recent attack. The absence of reaction in one examination is considered by us to in no way exclude typhoid infection. If the case remains clinically doubtful, the examination should be repeated within a few days.

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 has been found that about 20 per cent. gave 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. This peculiar property of the blood-serum may persist in persons who have recovered from typhoid fever for a number of months. Thus a definite typhoid reaction has been reported in serum from three months to a year, and a slight reaction, though much less than sufficient to establish a diagnosis of typhoid infection, from one to fifteen years, after convalescence from the disease.

The Reaction with the Blood-serum of Healthy Persons and of Those Ill with Diseases other than Typhoid Fever. An immediate marked reaction has not been observed in a 1 to 10 dilution of the blood-serum of over one hundred healthy persons examined in the health department laboratories. In several hundred cases of diseases, not believed by the physicians in charge to be at the end of the disease typhoid fever, only very rarely did the serum give a marked immediate reaction in a 1 to 10 dilution, and here in the light of past experience, I believe a typhoid infection, though not a typical typhoid fever, to have existed. These results have been confirmed by others, the question of dilution having been recently made the subject of elaborate investigations, with the view of determining, if possible, at what dilution the typhoid serum would react and others

would not. Thus, Schultz has reported lately that among 100 cases of non-typhoid febrile diseases apparently positive results were obtained in 19 with dilutions of 1 to 5, in 11 of these with 1 to 10, in 7 with 1 to 15, in 3 with 1 to 20, and in 1 a very faint reaction with 1 to 25; whereas in as many cases of true typhoid he never failed with dilutions of 1 to 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 to 25 dilution with a time-limit of two hours indicates less agglutinating substance than an immediate complete reaction with a 1 to 10 dilution.

From an experience with the practical application of the serum test for the diagnosis of typhoid fever extending over three 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, though not absolutely infallible, the Widal test is an indispensable aid to the clinical diagnosis of irregular or slightly marked typhoid fever.

The Isolation of Typhoid Bacilli from Suspected Feces, 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, feces, 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 in the main proved unsatisfactory, though the investigations of some of the later observers have given a number of positive results from the blood. The examination of the urine and feces of typhoid patients has more often given positive results than the blood, and these positive results have become more frequent and satisfactory as the methods for differentiating the bacillus typhosus have grown more exact and refined.

There are at present several recently devised media employed for the isolation and identification of the typhoid bacillus, which are much better than any of those formerly used. These are the Hiss, Capaldi, and Elsner media. In the hands of trained bacteriologists they give satisfactory results.

THE HISS MEDIA:¹ *Their Composition and Preparation.* Two 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.

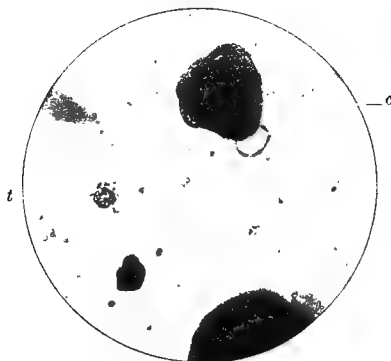
The plating medium is composed of 10 grammes of agar, 25 grammes of gelatin, 5 grammes of sodium chloride, 5 grammes of Liebig's beef extract, 10 grammes of glucose, and 1000 c.c. of water. When the agar is thoroughly melted the gelatin is added and

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 Semi-solid Culture Media," published in the Journal of Experimental Medicine, 1897, vol. ii. No. 6.

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.

The tube medium contains agar, 5 grammes; gelatin, 80 grammes; sodium chloride, 5 grammes; meat ex-

FIG. 54.



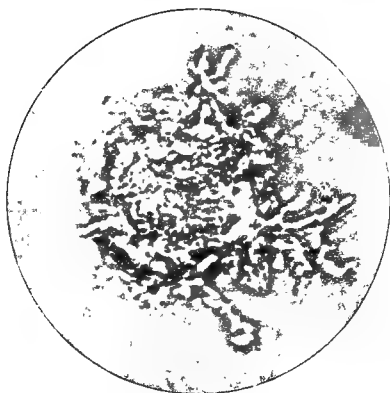
Hiss' plate media: Small light colony (*t*) is composed of typhoid bacilli; large colony (*c*) of colon bacilli. (From Hiss.)

tract, 5 grammes, and glucose, 10 grammes to the litre of water, and reacts to 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 (Figs. 54 and 55). The

FIG. 55.



Colony of typhoid bacilli more highly magnified. (Hiss.)

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 it 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 feces 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 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 sub-

jected 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.

ELSNER'S METHOD.¹ As Elsner himself gives no very definite details as to the steps to be taken in making up his medium, those working with it have developed their own modifications. We found the following method to give satisfactory results:

1. Grate 0.5 kilogramme of small potatoes to a fine pulp, add 1 litre of water, and allow the mixture to stand in a cool place over night.

2. Mash thoroughly (meat-press is best) and strain through a fine cloth. This must be done when the mixture is cold or the swelling of the starch-granules will prevent the filtering process.

3. Boil the filtrate and filter again.

4. Add 10 per cent. of gelatine and dissolve by boiling.

5. Test for the acidity. Elsner used litmus as an indicator, and advised that the medium be of such an acidity as to require the addition of 2.5 c.c. of decinormal hydroxide solution to make it neutral. If more than 2.5 c.c. are required the acidity must be reduced by normal sodic hydrate solution. Abbot advises using phenolphthalein as the indicator, and making the reaction such that 3 c.c. of the decinormal solution will neutralize 10 c.c. of the medium.

6. Boil and clarify with an egg.

7. Filter first through cotton and then through paper.

¹ Zeitschrift für Hyg. und Infek., 1896, Bd. xxi. S. 25.

8. To the filtrate add 1 per cent. of potassium iodide. (Use a solution so made that 1 c.c. is equivalent to 1 gramme of the salt.)

9. Decant into tubes and sterilize.

One of the most important points in working with this medium is that the incubator must be kept at a constant temperature of from 22° to 24° C. If the plates be put away before the gelatin has thoroughly cooled, or if the room becomes a very little too warm at any time during the colony growth, so as to soften the gelatin, both the typhoid and the colon bacilli will develop threads or become oval, and thus the characteristic differentiation will be lost. Care must be taken, also, that the room in which the plates are examined be not too warm. This causes great inconvenience during the summer months in most parts of the United States, and requires special methods for keeping a room at the proper temperature and keeping the plates cool during their examination.

The iodide of potash prevents the great majority of bacteria, especially the liquefiers, from developing; in fact, little but colon and typhoid bacilli appear on the plates. This is one of the chief advantages of the medium in the examination of both water and feces.

Appearance of the Colonies. The colon is the first to develop; the colonies are rough and granular in appearance and greenish-brown in color; for the greater part the colonies are on or near the surface. The typhoid develop later, and their colonies usually show the classical "dew-drop" appearance—small, white, gleaming, generally without variation in substance, but occasionally slightly granular. This point causes some trouble to one first using the medium, as the young colon colo-

nies sometimes present much the same appearance. However, it was found that the typhoid colonies usually grew near the surface, while the colon colonies, which are small and white, are almost invariably very deep. This is especially true after forty-eight hours; therefore, if one is not sure of a colony from the appearance, an attempt to "fish" it will usually identify it.

To one familiar with the medium, the characteristic appearance of a plate when typhoid is present is almost unmistakable, and it would seem that one would be almost sure to find the typhoid in some one of a series of plates, even if there were but few in the specimen.

Elsner says that the typhoids do not develop for forty-eight hours. Although the differentiation is more accurate after that time, still for practical methods of work twenty-four hours was found to be quite sufficient. When the plates are first taken from the incubator the diagnosis is not quite as certain, for the colon colonies will not have developed the characteristic color; but if the plates be allowed to stand in the light for a couple of hours the diagnosis will be found quite easy. After forty-eight hours all the large colonies will be colon and most of the small ones typhoid, if there be any typhoid present. Even after several days' standing in the ice-chest the typhoid colonies do not develop color.

Except for the difficulty in obtaining an exact temperature for growth and a cool room for examination, this method was found very satisfactory.

THE CAPALDI PLATE MEDIUM. In his original paper, Capaldi gives the following recipe:

Aqua dest.	1000
Gelatin	20
Mannite (grape-sugar)	10
Sodium chloride	5
Potassium chloride	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 the agar was added when the gelatin was put in in the original recipe, and the gelatin was added after the first filtration.

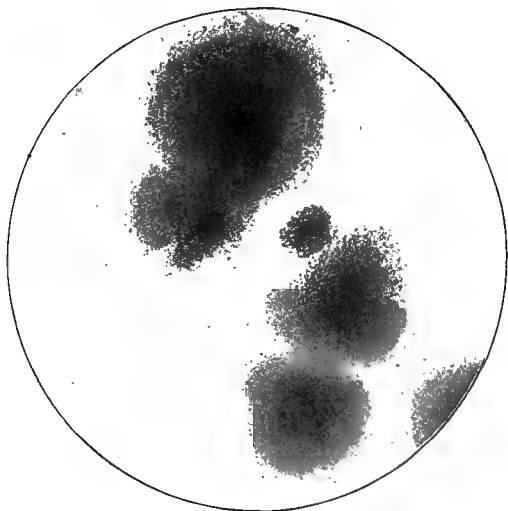
The Capaldi medium is usually employed for surface cultures, but can be inoculated while melted in the tubes. Plates may be made beforehand, so that they are ready for use when the specimen comes in. As these plates are to be kept at 37° C., the difficulties in regard to temperature are avoided; but, unlike the Elsner plates, other organisms beside 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 feces or urine. Others are then inoculated with a loop of urine or much diluted feces. In this way we are apt to have some plates with just the right amount of colonies.

Appearance of the Colonies. Capaldi thus describes the differentiation: Typhoid: Small, gleaming, 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 (Fig. 56). However, it is often transparent, without the refractive granules; sometimes with a nuclear centre, and sometimes of equal consistency throughout. Streptococci simulate typhoid, but a high-power lens will show the coccus.

FIG. 56.



Colonies of typhoid bacilli, on Capaldi medium, slightly magnified.

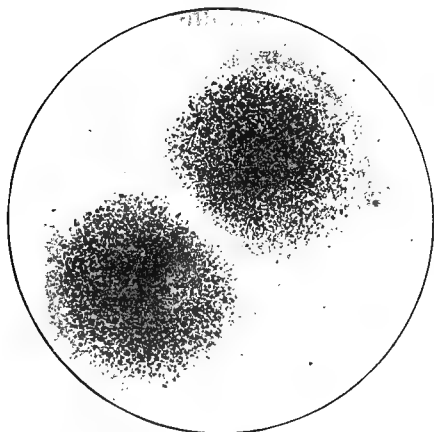
Colon colonies are usually much larger than the typhoid; a decided brown color, very large, refractive granules, and in general quite different in appearance (Fig. 57).

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.

The two media together (Capaldi and Elsner) work excellently, as one is an aid to the other. When many colonies of the typhoid bacilli were present the

FIG. 57.



Colonies of colon bacilli. Capaldi medium slightly magnified.

differentiation was 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.

As to the comparative merits of these 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, if they are present, except perhaps when they

exist in only the most minute numbers. The Elsner method has the objection that it is very difficult to work with in hot weather. 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 varieties of the typhoid bacillus do not produce typical thread outgrowths from the colonies.

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 Capaldi medium for the plate cultures and the Hiss tube medium for identifying the bacilli obtained. Through these media we are now in a position to obtain and identify typhoid bacilli from feces, urine, etc., within forty-eight hours.

Recently numerous investigations have been carried out to discover how frequently and at what period in typhoid fever cases bacilli were present in the feces and urine. In the laboratory Hiss has recently examined the feces in forty-three consecutive cases, thirty-seven of which were in the febrile stage and six 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 in hospitals examined, 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 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 after the defervescence, have been confirmed by others. In several cases in which no Widal reaction was demonstrated the bacilli were isolated. From private sources between the seventh and twenty-first day of the disease, experience thus far obtained 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 feces 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 feces and upon its chemical formation. Probably the presence of typhoid bacilli in some stools and their absence in others must be explained largely upon the characteristics of the intestinal contents. The short life of the typhoid bacillus in many specimens of feces suggests that stools be examined as quickly as possible.

In fact, unless the physician wishes to take the trouble to have the sample of feces 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 even more interest than the presence of the bacilli in feces is their frequent occurrence in great numbers in the urine. The results of the examinations of others as well as those of 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, and usually in pure culture and in enormous numbers. Of nine positive cases examined by Richardson¹ two died and seven were discharged. At the time of their discharge their urine was loaded with typhoid bacilli. We have noted similar cases. In one the bacilli persisted for five weeks. Undoubtedly in some cases they persist for months. 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 feces 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.

¹ Journal of Experimental Medicine, May, 1898.

The Detection of Typhoid Bacilli in Water. This subject is considered on pages 247 and 248. There is absolutely no doubt that the contamination of streams and reservoirs is the 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 owing to the contamination often having occurred and passed away before the bacteriological examination is undertaken, and also because of 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 which is thrown into the water, and the shorter the time which elapses between the infection and the drinking of the water, the greater is the danger.

The typhoid bacillus and the colon bacillus of Escherich resemble each other in many respects. The characteristics of each, which allow us to differentiate the one from the other, will be considered at the end of the description of the colon bacillus.

CHAPTER XXIV.

BACILLUS COLI COMMUNIS (OR COLON BACILLUS OF ESCHERICH).

THIS organism was first described by Emmerich (1885), who obtained it from the blood, various organs, and intestinal discharges of cholera patients at Naples. It was afterward found by Escherich (1886) in the feces of healthy milk-fed infants and by Weisser in the alvine discharges of healthy men. It has since been demonstrated to be a normal inhabitant of the intestines of man and of many of the lower animals.

Morphology. The size and shape of the bacillus coli varies considerably in its morphology according to the sources and the culture media from which it is obtained. The typical form is that of short rods with rounded ends, from 0.4μ to 0.7μ in diameter by 1μ to 3μ in length; but sometimes 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 (Fig. 58). The bacilli may occur as single cells or as pairs joined end-to-end, rarely as short chains. In unfavorable culture media in stained preparations they may present unstained spaces (vacuoles) and more intensely stained portions at the extremities, closely resembling spores, but these are due, according to

Escherich, to degenerative changes in the protoplasm. The colon bacillus does not form spores. There is nothing in the morphology of this bacillus which is characteristic or which may aid in its identification, for in this respect it simulates many other organisms.

FIG. 58.

Colon bacilli. Twenty-four-hour agar culture. $\times 1100$ diameters.

The colon bacillus *stains* readily with the ordinary aniline colors; it is quickly decolorized by Gram's method.

Biology. It is an *aërobic, facultative anaërobic, non-liquefying* bacillus. It is motile, but its movements are so sluggish that a positive opinion is often difficult, being exhibited often by one or two individuals, in fresh cultures, and at a high temperature only. These movements are produced by flagella, which may be demonstrated by Löffler's method of staining, though not usually in such numbers as are seen to occur on the bacillus typhosus.

Growth on Gelatin. In gelatin plates colonies are developed in from twenty-four to forty-eight hours, which vary considerably in their appearance according to their age, and in different cultures in the same medium. They resemble greatly the colonies of the typhoid bacillus, except that they are somewhat larger for the same period of growth. 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, in structure round, 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." The superficial colonies appear as small, dry, irregular, flat, blue-white points that are commonly somewhat dentated at the margin.

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.

On Nutrient Agar and Blood-serum. On nutrient agar and blood-serum an abundant, soft, white layer is quickly developed in the incubator, but the growth is not characteristic.

In Bouillon. In bouillon the bacillus coli produces diffuse clouding with sedimentation; in some cultures a tendency to pellicle formation on the surface is seen occasionally. In old cultures, in the absence of sugar, the reaction becomes alkaline, and a decided fecal odor may be noticed.

The colon bacillus produces indol in bouillon and in peptone solutions, this reaction being most pronounced after a week's development in the incubator. It pos-

sesses also a considerable reducing power, converting nitrates into nitrites, as may be demonstrated by the addition of sulphuric acid in the proper proportion to a bouillon or peptone culture, when a pink coloration results.

On 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 on potato; there may be no growth at all, or it may be scanty and of a white color. These variations are due at times to the bacillus, but more often to variations in the potato.

Gas-production. The bacillus coli grows rapidly in media containing glycerin and sugar, particularly glucose, causing active fermentation with liberation of carbonic acid and hydrogen gas. Cultivated in solid media, to which glucose has been added, the gas-production is recognized by the appearance of numerous bubbles along and about the points of growth; in fluid media it may be demonstrated in the fermentation-tube. Grown on lactose-litmus-agar, the colonies are pink and the color of the surrounding medium is changed from blue to red, showing the production of acid.

Milk is coagulated by the growth of the bacillus coli after twenty-four to seventy-three hours in the incubator, with the production of gas and acid; very rarely acid may be produced and no coagulation occur. The coagulation of the milk is hastened by warming.

The thermal death-point of the colon bacillus from feces was found by Weisser to be 60° C., the time of exposure being ten minutes. When the bacilli from

a bouillon culture were dried upon thin glass covers they failed to grow after twenty-four hours (Weisser). Waliczek found that when dried upon pieces of sterile filter-paper they failed to grow at the end of eighteen hours. These results give confirmation to the view that the colon bacillus does not form spores.

Pathogenesis. The colon bacillus is pathogenic in varying degrees for test animals, though the results of the inoculations, as with the typhoid bacillus, cannot always be predicted with certainty. Intraperitoneal injections of from 0.1 to 1 c.c. of fresh, virulent cultures usually produce death in mice at the end of from one to eight days, but death does not invariably follow. The more rapidly death ensues the greater the number of bacilli found in the body; they are always more abundant in the abdominal cavity than in the blood; in other words, the result is to be attributed to the toxic rather than to the infective properties of the culture used. But the fact that the bacilli are found in the blood and internal organs when death rapidly follows inoculation proves that they do multiply to some extent in the body. When less virulent cultures, however, are injected and death results, this is due to the poisonous products formed by the bacilli and given up at their death. The lesions produced are those of enteritis: the duodenum and jejunum are found to contain fluid, the spleen is somewhat enlarged, and there is marked hyperæmia and ecchymosis of the small intestines, together with swelling of Peyer's patches.

Intraperitoneal and intravenous inoculation of guinea-pigs and rabbits may also 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 fibro-purulent peritonitis.

When subcutaneous inoculations of mice and guinea-pigs are made it requires the introduction of much larger quantities of the culture to produce infection; in rabbits this is followed only by abscess formation at the point of inoculation. Dogs and cats are similarly affected.

Bazy and Guyon have succeeded in producing infection of the bladder in animals by injection of pure cultures into the blood with simultaneous tying of the ureters; Albaran 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. Loruelle, Fraenkel, and Barchacci, by injuring or tying the intestines and introducing dirt into the abdominal cavity, with or without the simultaneous injection of cultures of the colon bacillus, succeeded in causing diffuse peritonitis in animals. Akermann produced osteomyelitis in young rabbits by intravenous injections of cultures. So far all attempts to produce experimental infection of the intestines by the ingestion of cultures of the colon bacillus have failed to give positive results (Emmerich and Korkunoff).

Certain peculiar effects have been observed by Blackstein and by Gilbert and Lion as the result of intravenous inoculation of rabbits with pure cultures of the bacillus coli, which are worthy of note. The former of these authors found, from eight to thirty-eight days after injection, that the liver frequently contained opaque, whitish or yellowish-white spots, and streaks

of irregular size and shape, giving a peculiar mottling to the organ when present in large numbers. By microscopical examination these were found to represent places where the liver cells had undergone necrosis, accompanied by emigration of leucocytes, and the cells about them were in a condition of fatty degeneration. In sections of the liver, masses of the bacilli were discovered in and about the necrotic foci. The bacilli were not found generally distributed through the body, but only in the bile, liver, and occasionally in the spleen. Gilbert and Lion found in addition that hemiplegia and paraplegia were often produced in consequence of atrophy of the cells of the cord. These observations have been confirmed by Thoinot and Massilin, but in their experiments the nerve-lesions were not commonly present.

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 products of the organism rather than in its mechanical presence in the tissues.

Variation in Virulence. The virulence of the colon bacillus varies considerably as derived from different sources. An attempt has been made to establish certain rules for this. Thus, Lesage and Macaigne express the opinion that when obtained from a healthy body it is only slightly virulent, while that isolated from a diseased person is much more virulent. The infective power is thought to bear a definite relation to the severity of the disease with which the organism is associated; for instance, to be greatest in cultures taken from cholera patients and least in those obtained from pus. Dreyfus also confirms this view. He found

by experiment that 1 c.c. of a fresh bouillon culture of the *B. coli* from normal feces was required to kill guinea-pigs by intraperitoneal and rabbits by intravenous injection, whereas less than one-fifth as much of a culture from a fatal case of cholera nostras was sufficient to kill the same animal; but this rule has probably many exceptions, even if it be true in some cases.

All observers, however, agree that the virulence of the *B. coli* is diminished by continued cultivation through successive generations, and that it is increased by passage through animals.

Immunization. Immunization against colon infection is comparatively easy to produce in the usual way by the inoculation of gradually increasing doses of cultures of the living bacilli or dead bacilli.

Occurrence in Man and Animals. The bacillus coli communis is a common inhabitant of the intestinal canal in man and in many animals. According to Fremlin, it is found normally in dogs, mice, and rabbits, but not in rats, pigeons, or guinea-pigs. According to Dyas and Keith, it occurs in goats, rabbits, dogs, cats, swine, and cows, but not in horses. Grimbart claims to have found it in the intestines of almost all domestic animals, and in the mouth as well as the intestines of man. It is also frequently found in water and food (milk, etc.), so that it is one of the most widespread saprophytic bacteria known. Formerly it was thought that the presence of the *B. coli* in water was sufficient proof of its contamination by feces; but the recent investigations of Weichselbaum, Kruse, Beckmann, and Refith would seem to show that there are no grounds for this assumption, as the colon bacillus may reach the water from many different sources.

From its common seat in the intestines it may, under favoring conditions, penetrate other organs after death—which fact may account for its being found so often at autopsy in the interior of the body; but it may also be absorbed during life, more especially if there is obstruction of the intestines or if the mucosa has been deprived of its epithelium. For this reason, no doubt, the *B. coli* is so frequently found in cholera, typhoid fever, and dysentery, producing often a second infection. The absorption of the colon bacillus from the intestinal canal plays an important part, probably, in the production of many diseases, such as cystitis and other inflammatory affections. It has been considered to be the cause of epidemic infectious enteritis and cholera nostras, this assumption being based upon the facts that the colon bacillus in these diseases is found in greater abundance than usual in the alvine discharges and often in pure culture; that it then possesses an increased virulence, and that it often penetrates the interior organs, as has been shown by autopsies. But the conclusion drawn from these facts as to the etiology of the diseases above mentioned is not positive, though it cannot be denied that under certain conditions the colon bacillus may be productive of disease. This is brought about, according to the commonly accepted view, either by an increase of virulence of the *B. coli* normally present in the intestines or by the introduction of especially virulent bacilli in the food. The colon bacillus has also been assumed to be the cause of cholera infantum; but the investigations of Booker, Baginsky, Escherich, and Flügge would seem to indicate that this disease is of a much more complicated origin. The *B. coli*, moreover, is associ-

ated with dysentery, probably as a secondary affection, as in amœbic or tropical dysentery. It is also found frequently in cases of diffuse and circumscribed peritonitis, appendicitis, etc., either alone or together with other bacteria which play a part in the etiology of these diseases along with certain chemical ferments and toxins and foreign bodies in the intestines. The origin of infections of the gall-ducts (with at times the production of gallstones) and multiple abscess of the liver is also explained in this way by Dmochowski and Janowski, though, according to Letienne, the mere presence of the *B. coli* in the bile, in which it has been found under normal conditions, is not sufficient to account for these affections. Puerperal fever is not infrequently caused by the colon bacillus by infection of the vagina or uterus. Other diseases to which the colon bacillus seems to stand in a certain relation, though rarely, are: Endocarditis, meningitis, tropical abscess of the liver, bronchopneumonia and an irregular type of lobar pneumonia, fetid bronchitis, chronic amygdalitis, and abscess of the lachrymal sac. The *B. coli* has been found in a case of urethritis (pseudogonorrhœa) lying inside the cells like gonococci, and it is often associated with the pyogenic cocci in cutaneous and subcutaneous purulent inflammations.

In the above-mentioned diseases the colon bacillus has been found either alone or associated with other pathogenic bacteria in such numbers as to be considered a factor in the etiology of the disease, and in some cases there is no reason to doubt that it is the primary cause of infection. Though further study and investigation are required to show the specific pathogenic properties of this micro-organism, it is evident

that under certain conditions it may become pathogenic to man.

According to many authorities there are a great number of varieties of the colon bacillus, some maintaining even that it may become, under suitable conditions, identical with the typhoid bacillus; but there has been no proof whatever of this.

Differential Diagnosis. 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 conspicuous than that of the *B. typhosus*. It 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 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.

7. The colon bacillus possesses the property of producing indol in cultures of bouillon or peptone; the typhoid bacillus in these solutions does not produce indol, except in a few rare exceptions.

8. The colon bacillus rarely produces thread outgrowths in the 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. On the Elsner medium the colon colonies appear earlier and become larger and more opaque than the average typhoid colonies.

11. Finally, we have the test of placing the bacilli in animals and in the hanging drop, together with the serum of animals immunized to either the colon or the typhoid bacillus.

None of these tests alone can be depended upon for making a differential diagnosis of the colon bacillus from the typhoid bacillus or other similar bacilli.

Unfortunately in most, at least, of these characters certain degrees of variation may often be observed in different cultures of the typhoid and colon bacillus which may lead to confusion. For instance, the mor-

phology may vary considerably, even at times when grown on the same culture media, and the motility is not always equally active; the flagella formation 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; the indol test requires great care in its performance, and in rare instances the typhoid bacillus produces it; the growth on potato is not to be depended on, being often 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 absolutely infallible in all cases, for once we met with a bacillus in feces which grew in a manner utterly at variance with the typhoid bacillus, yet still gave the Widal reaction perfectly with the serum of an immunized horse. It is also stated by Abbott that all typhoid bacilli do not give the Widal reaction with the serum derived from a typhoid infection with a single variety of a typhoid bacillus. This is an experience that as yet we have not met with. The Pfeiffer reaction in guinea-pigs is a matter of extreme delicacy, and varying results are sometimes obtained.

In spite, however, of these difficulties it is very easy to sufficiently identify the typhoid bacillus for all practical purposes. A bacillus which grows typically in the Hiss tube media and shows the Widal reaction with a high dilution of the serum of an animal immunized

to the typhoid bacillus, is in all probability the typhoid bacillus. The same could probably be stated of a bacillus which grew characteristically in glucose bouillon and nutrient gelatin, and also showed the specific serum reaction. Probably not one time in ten thousand would such bacilli prove on further investigation not to be typhoid bacilli. 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 agglutinates typhoid bacilli.

CHAPTER XXV.

PNEUMOBACILLUS ; FRIEDLÄNDER'S BACILLUS.

DISCOVERED by Friedländer (1883), and declared by him to be the cause of fibrinous pneumonia. Subsequent researches have shown that it is present in only a small proportion of the cases of this disease. It is found also not infrequently in the mucous membranes of the mouth and air-passages of healthy individuals, and in the air.

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.

Biological Characters. 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. Gas-bubbles occasionally develop in gelatin, 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 and culturally. 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. On *blood-serum* abundant, grayish-white, viscid masses are developed. 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. *Fermentation* of milk-sugar and glucose is caused. 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. In Friedländer's experiments mice proved to be particularly susceptible. These animals, when pure cultures of the bacillus are injected through the thoracic wall into the tissue of the lung, invariably succumb to the disease. On autopsy the pleural cavities are found to contain a sero-purulent 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. In guinea-pigs which are killed by the inoculation similar appearances are observed.

Friedländer's bacillus has been found in man, not only in patients suffering from croupous pneumonia and other respiratory diseases, but also in healthy indi-

viduals, and in the outside world. Thus, Pawlowsky found it in the atmosphere and Mori in canal water; 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. Friedländer believed that the bacillus described by him was the specific cause of croupous pneumonia; but in 129 cases examined by Weichselbaum this bacillus was found in only 9; of 70 cases examined by Wolf only 3 showed the presence of Friedländer's pneumobacillus. It is evident, therefore, that though this micro-organism may be concerned in the production of certain forms of the disease, it is not the specific cause of croupous pneumonia. The cases which are due primarily to the pneumobacillus are distinguished, according to Weichselbaum and Netter, by their peculiarly malignant type and by the viscosity 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 diseases it has been found at times to be present.

CHAPTER XXVI.

THE PRODUCERS OF ABSCESS, CELLULITIS, SEPTICÆMIA, ETC.

THE STAPHYLOCOCCI. THE MICROCOCCUS TETRAGENUS.

The *Staphylococcus Pyogenes Aureus*. (The Golden *Staphylococcus*.)

THE *staphylococcus pyogenes aureus* is one of the commonest pathogenic bacteria, being almost everywhere present, and is the organism most frequently concerned in the production of acute, circumscribed, suppurative inflammations. It was first observed by Ogston (1881) in the pus of acute abscesses, but was not obtained by him in pure culture. It was isolated from the pus of acute abscesses and accurately described by Rosenbach (1884).

Morphology. Small, spherical cells, having a diameter of 0.87μ (Passet), occurring solitary, in pairs as diplococci, in short chains 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. 59.)

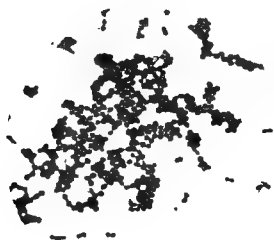
It *stains* quickly in aqueous solutions of the basic aniline colors. When previously stained with methyl-violet it is not decolorized by Gram's method.

Biology. The *staphylococcus pyogenes aureus* is an *aërobic, facultative anaërobic, liquefying* micrococcus,

growing readily at a temperature from 18° to 20° C., but best at 37°, in milk, bouillon, and other liquid media, and in nutrient gelatin or agar, accompanied by liquefaction of the gelatin.

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

FIG. 59.



Staphylococcus. $\times 1100$ diameters.

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 stick 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 "golden staphylococci" form a golden-yellow or orange-colored deposit at the bottom of the tube. Under unfavorable conditions the staphylococcus aureus gradually loses its ability to make pigment and to liquefy gelatin.

Growth on Agar. In streak and stick cultures on agar a whitish growth is at first produced, and this at the end of a few days becomes 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 inoculated with this micrococcus at the end of from one to eight days is coagulated; *bouillon* and *peptone* solutions are densely clouded by the luxuriant growth produced.

In the three last-named culture media, as the 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.

The staphylococcus is distinguished from most other pathogenic bacteria by its comparatively 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. Its thermal death-point is

between 56° and 60° C., the time of exposure being ten minutes (Sternberg). Bolton found that a 1 per cent. solution of carbolic acid destroyed the vitality after two hours' exposure. Mercuric chloride, 1 : 1000, destroys it in from five to ten minutes, according to most authorities, though Abbott found that in the same culture there may be a considerable difference in the resisting power of the cocci, all being frequently destroyed in five minutes, while, again, some may survive after an exposure to a solution of 1 : 1000 for ten, twenty, and even thirty minutes.

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 the experiments so far made this micrococcus, as found in suppurative processes in the human subject, has not proved to be as infectious for animals as it is for man. 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 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. 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 sub-

ject, 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 heal generally 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, after injuring the cardiac valves in rabbits, produced ulcerative endocarditis. Subsequently, Weichselbaum, Prudden, and Fraenkel and Säger 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 was 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. With regard to the lesions found in the kidneys after intraperitoneal or intravenous inoculation of cultures of the staphylococcus, it has been found that when injected in considerable quantities the organism may be obtained in cultures from the urine, but not sooner than six or eight hours after the injection, and not until the formation of purulent foci in the kidneys has already occurred.

The Production of Toxic Substances. The peculiar energetic action of the staphylococcus pyogenes aureus on the tissues of warm-blooded animals would seem to indicate that toxic substances are produced by this organism, which play an important part in its infective properties. Grawitz and De Bary have

shown by experiments that cultures of the staphylococcus, when sterilized by boiling and injected subcutaneously into dogs, will produce 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 insensible, and perforation alongside of the sclerotic ring finally taking place, followed by the formation of pus in the anterior chamber and recovery. These local changes are the results of 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. Dogs and guinea-pigs thus treated usually die, showing symptoms of poisoning. From the bodies of the bacteria Leber obtained, by treating them with alcohol and ether, a crystalline, chemical substance, which he called *phlogosin*. This substance, which is an energetic pus-producer, is supposed to be the active principle of the staphylococcus aureus.

Immunization. Immunity against staphylococcus infection may be produced in different animal species by the injection of increasing doses of the pure culture, either living or previously sterilized by boiling. Reichel thus succeeded in immunizing dogs against a surely fatal dose of living as well as dead staphylococci. Viquerat claims to have immunized horses in the same way.

The blood-serum of animals which have been immunized by means of living or dead cultures possesses slight immunizing and curative effects in other animals, but no practical use of the serum has been attempted in man.

Occurrence in Man. The *staphylococcus pyogenes aureus* is the commonest pyogenic micro-organism found in man. From the fact that these micrococci are so constantly present in the pus of acute abscesses, as demonstrated by Ogston, Rosenbach, Passet and others, it was formerly assumed that there could be no pus-formation in the absence of micro-organisms of this class; but it is now well known, from the experiments, that certain chemical substances, such as nitrate of silver, oil of turpentine, strong liquor ammoniæ, etc., introduced beneath the skin, give rise to pus-formation quite independently of bacteria. Practically all micro-organisms, moreover, 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 extended researches of bacteriologists show that few species are usually concerned in the production of acute abscesses, furuncles, etc., in man. Of these the two most important, by reason of their frequent occurrence and pathogenic power, are *staphylococcus pyogenes aureus* and *streptococcus pyogenes*; next to these comes the *staphylococcus pyogenes albus*. Two or more species 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.

As the result of extended researches, however, made by bacteriologists within recent years the golden *staphylococcus* has been demonstrated not only in furuncles and carbuncles, but also in various pustular affections

of the skin and mucous membranes—impetigo, sycosis, phlyctenular conjunctivitis; in purulent conjunctivitis and inflammation of the lachrymal 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; in infectious osteomyelitis, in ulcerative endocarditis, pyelonephritis, etc. It is one of the chief etiological factors in the production of pyæmia in the various pathological forms of that condition of disease.

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 seat, 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 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. Bockhart rubbed upon the uninjured skin of the forearm a small quantity of an agar culture suspended in salt solution. By gently scratching with a disinfected finger-nail the epithelium was removed in places over the area to which the micrococcus had been applied. Numerous impetigo pustules, and occasionally a genuine furuncle, developed as the result of the procedure. Bockhart examined portions of the skin, which he excised for the purpose, under the microscope, and came to the conclusion that the cocci penetrate by way of the hair-follicles, the sebaceous and sudoriparous glands, or, where the epidermis had been removed by scratching, directly to the deeper layers of the skin.

Staphylococcus Pyogenes Albus.

Isolated by Rosenbach (1884) from the pus of acute abscesses, in which it is sometimes the only micro-organism present, and sometimes associated with the staphylococcus aureus and other pyogenic cocci.

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. 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.

According to Passet, it is more common than the aureus in man; but 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 Epidermis Albus (Welch).

Probably identical with the staphylococcus pyogenes albus, but found by Welch on the surface of the body, though often present in parts of the epidermis deeper than can be reached by any known means of cutaneous disinfection save the application of heat.

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 a nearly, if not quite, constant inhabitant of the epidermis. It is now clear why I have proposed to call it the staphylococcus epidermis 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 Bossowski and 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 aureus not only in color, but also in the fact that it liquefies gelatin more slowly, does not so

quickly cause coagulation in milk, and is far less virulent when injected into the circulation of rabbits. It has been shown by the experiments of Bossowski and of Welch that this micro-organism 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.

Isolated by Passet (1885) from the pus of acute abscesses, in which it is occasionally found (about 10 per cent. of the cases examined) in association with other pyogenic cocci. It is morphologically identical with the staphylococcus aureus and albus, being distinguished from the other species only by the formation of a lemon-yellow pigment instead of a golden-yellow, as in the aureus, and a white or colorless deposit, as in the albus.

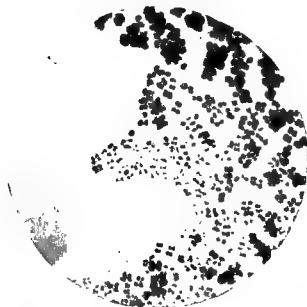
THE MICROCOCCUS TETRAGENUS.

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 ab-

scesses. 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 directions, 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, undivided cells, in pairs of oval elements, and in groups of three and four (Fig. 60). When the divis-

FIG. 60.

Micrococcus tetragenus. $\times 1000$ diameters.

ion 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.

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.

Biological Characters. 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 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 colonies may be either isolated or confluent, in the case forming a thick, white, slimy mass, filling out the fissures and hollow spaces all along the line of puncture; on the surface a broad, thick layer of 4 to 5 mm. in extent is apparent. The gelatin is not liquefied.

Growth on Agar and Blood-serum. On plate and slant cultures of agar and blood-serum the surface of the growth is moist and glistening. 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 animals remain apparently well for a day or two, then become quiet, until death takes place on the third or sixth day. 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. Gray mice are, for the most part, immune to infection by the micrococcus tetragenus. Guinea-pigs at times show only a local reaction after inoculation, and again die from septicæmic infection. When intraperitoneal injections are

given they are followed by purulent peritonitis, beautifully formed cocci in groups of four being obtained in immense numbers in 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.

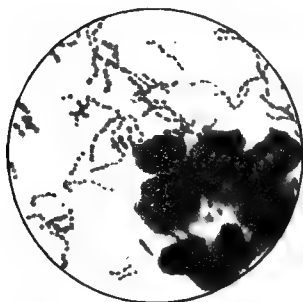
CHAPTER XXVII.

STREPTOCOCCUS PYOGENES (STREPTOCOCCUS ERYSIPELATUS ; STREPTOCOCCUS OF PUS ; STREPTOCOCCUS PATHOGENES LONGUS).

THIS micrococcus was first observed by Koch in stained sections of tissues 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 the streptococci growing in long chains are but variations of one and the same

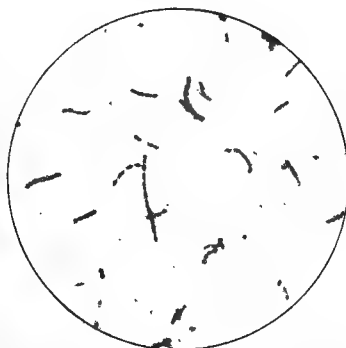
species which has been appropriately termed the "streptococcus pathogenes longus." Some of the streptococci, at least in so far as their specific products and their reaction in the presence of a curative serum is concerned, belong to a species as distinct from the streptococcus pyogenes as the pneumococcus. This question has a very practical side, for upon its decision rests our ability to choose a suitable protective serum in cases of streptococcus infection.

FIG. 61.



Streptococci in peritoneal fluid, partly enclosed in leucocytes. \times 1000 diameters.

FIG. 62.



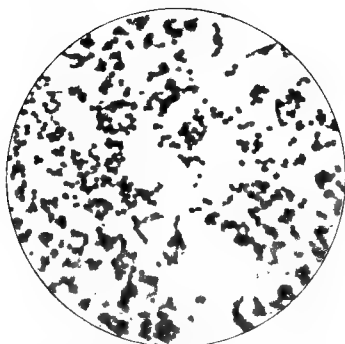
Streptococci in throat exudates smeared on cover-glass. \times 1000 diameters.

Morphology. Spherical cocci, when fully developed, having no independent movements, from 0.4μ to 1μ in diameter, usually larger than the staphylococci, but varying 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 certain media the cocci occur mostly in diplococci, but usually they grow in longer or shorter chains. Certain cocci fre-

quently exceed their fellows greatly in size, especially in old cultures, when this may be considered to be the result of involution forms. (See Figs. 61, 62, 63, and 64.)

They *stain* readily by aniline colors and by Gram's method.

FIG. 63.



Streptococci from solidified serum culture appearing mostly as diplococci. $\times 1000$ diameters.

FIG. 64.



Streptococcus growing in long chains in bouillon culture. $\times 1000$ diameters.

Biological Characters. 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 freely at ordinary room-temperature— 18° to 20° C. They are facultative anaërobes, growing both in the presence and absence of oxygen.

Growth on Gelatin. Tubes of gelatin which have been inoculated with streptococci by puncture with the 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*, though occasionally, with unusual varieties, a certain amount of liquefaction has been observed to take place.

Growth on Agar. On agar plates the colonies are visible after twelve to thirty hours' growth, and present a beautiful appearance when magnified sufficiently to see the individual cocci in the chain. The colonies from different sources 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. The colonies are nearly circular in shape when thinly scattered over the plates, but irregular in form when crowded together.

Growth in Bouillon. Streptococci grow readily in slightly alkaline 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 the 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.

The development in a mixture of ascitic fluid and bouillon, which is the best medium for the growth of the streptococcus, is more abundant than in bouillon. The liquid is clouded, and a precipitate only occurs after some days, the fluid gradually clearing.

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, coagulation of the casein occurs with the production of acid, but this is not always the case.

The Duration of the Life of Streptococci 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 bouillon cultures they are usually short-lived, the majority dying within two or three days, and very few living over a month; but in serum bouillon they live much longer. In order to keep streptococci alive and virulent, it is best to keep them in serum or ascitic fluid bouillon in small, sealed glass tubes in the ice-chest. The thermal death-point of the streptococcus, according to Sternberg, is between 52° and 54° C., the time of exposure being ten minutes.

Von Lingelsheim has reported the following results obtained in an extended series of experiments made to determine the germicidal power of various chemical agents as tested upon this micro-organism (time of ex-

posure, two hours): Mercuric chloride, 1 : 2500; sulphate of copper, 1 : 200; trichloride of iodine, 1 : 750; peroxide of hydrogen, 1 : 50; carbolic acid, 1 : 300; cresol, 1 : 250; lysol, 1 : 300; creolin, 1 : 130.

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 may be 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 even 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, until recently through the use of specific serum, have 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 previous classification of streptococci, 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 the 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 are the most dangerous to any animal which have come immediately from septic conditions in the same species of animal, and the more virulent the case the more virulent the streptococci are apt to be in other 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 (Knorr, Petruschky). If the culture does not have the required virulence to produce this effect the virulence can usually be acquired by passage through animals. Streptococci obtained from the same disease and from the same individual usually show very much the same degree of virulence.

Occurrence in Man. Streptococci have been found in man as the primary cause of infection in the following diseases: Erysipelas, acute abscesses, small and large, cellulitis, circumscribed as well as diffused, sepsis, puerperal infection, lymphatic abscesses, angina, pneumonia, periostitis, otitis media, mastoiditis, meningitis, empyema, and endocarditis. Associated with other bacteria in diseases of which they were the specific cause, they have also been found as the secondary or mixed infection in many diseases, such as in pulmonary tuberculosis, bronchopneumonia, septic diphtheria, and diphtheritic scarlatina. 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 Löffler 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 streptococci present in the pseudo-membranous inflammations of patients sick with scarlet

fever that many investigators have suspected them to be the cause of this disease (Kurth, Baginsky, Roskin). They are found, however, regularly in the secretion of healthy individuals (in 100 examinations by us we found them in 83, and probably could have found them in the others by longer search). Their presence in scarlet fever is most probably due to their increase in the disordered mucous membrane.

The causal relation of the streptococcus to the above-mentioned diseases has been amply proved by inoculation experiments both in man and animals. Fehleisen has 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 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, and in some cases of sarcomata, with considerable success. In carcinomata the results have been very slight. In this country the experimental work upon this subject and the actual treatment of

cases has been largely carried out by or under the direction of Dr. 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, and in no case has the disease remained in abeyance sufficiently long to be regarded as cured.

“ 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 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 unquestioned disappeared, and the patients have remained well beyond three years. Nearly half of the cases treated showed 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 has 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.

“It is a curious fact, from the stand-point of pathology, that the largest percentage of successful cases has occurred in the spindle-celled variety, the very one in which errors of diagnosis are practically impossible. In addition to microscopical examinations by the best of pathologists, the malignancy of the tumors was further confirmed by the characteristic clinical appearances, and in many cases by a history of repeated recurrences.

“I have now three cases of spindle-celled sarcoma which have remained well beyond three years; one case of mixed (round and spindle) celled, which, after remaining well three and one-fourth years, had a return in the abdomen, and died about eight months later. This case certainly would establish the correctness of the early diagnosis.”

Dr. Coley would be the first to acknowledge that even the very moderate claims put forward in this communication are disputed by many surgeons, they claiming that the disappearance of the tumors is due to other causes than the treatment. In spite, however, of the treatment being frequently deleterious to the general health, and the occurrence from time to time of the spontaneous disappearance of apparently malignant tumors, I think we must allow that the proof is very strong that some sarcomatous tumors have been arrested and caused to disappear by the toxin injections, and that where they are clearly inoperative and progressing the treatment should be tried.

Production of Toxic Substances. There is no doubt that this micrococcus causes fever, general symptoms of intoxication, and death by means of toxic substances which it forms in its growth; but what these substances are—whether they are due to splitting up of animal proteids, or are secretion-products, or whether they are contained in the cell-bodies of the organism—what their composition is and how they are produced in cultures we do not know.

Susceptibility to Streptococcus Infection. As with the other ever-present pus cocci, the staphylococci, which have, as a rule, only slight virulence, the streptococcus is 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 or sequelæ of various specific infectious diseases, in the victims of chronic alcoholism, and constitutional affections in those exposed to septic emanations from sewers, etc., and probably in many cases from the absorption of toxic products formed in the alimentary canal as a result of the ingestion of improper food, or of abnormal fermentative changes in the contents of the intestine, or from constipation, are to be explained.

Immunity. Knorr succeeded in producing a moderate immunity in rabbits against an intensely virulent streptococcus by injections of very slightly virulent cultures. Pasquale was able to immunize these animals partially against septicæmia. Marmorek has immunized sheep, asses, and horses against very large

doses of a streptococcus, which though but slightly virulent for them was intensely so for rabbits.

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 days to months, tend to recover, and to a certain extent, therefore, we may assume protective processes have been called forth. 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 to again progress and that to perhaps 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. One method is now chiefly used for the immunization and attempt to produce curative substances in animals. The living, virulent streptococcus itself is injected in gradually increasing doses. Marmorek¹ was the first to attempt to produce a curative serum on a large scale.

Influence of Serum from Animals Immunized Against Streptococcus Infection upon Streptococcus Infections in Other Animals.

The results reported since Marmorek's communication in 1895 upon the immunizing effects of anti-streptococcic serum in animals have been very variable.

Reliable positive results are, however, more important than negative ones, since they indicate under proper conditions what can be accomplished. This is certainly true if at the same time we can find good reasons for the failures reported.

For the data in the following table I am indebted to Anna W. Williams, assistant bacteriologist in the Health Department Laboratories. For the use of the same I wish to express my appreciation.

In this present 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. In the following experi-

¹ Annales de l'Institut Pasteur, July, 1895.

ments 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 ANTI-STREPTOCOCCIC SERUM GIVEN BY SELECTED HORSES AFTER SIX MONTHS OF INJECTION OF SUITABLE AMOUNTS OF LIVING STREPTOCOCCI.

	Weight of rabbit.	Amounts inoculated.		Result.	Autopsy.
Serum and culture :	Grms.	Serum.	Cult.		
1. Inoculated at same time	1430	0.25 c.c.	0.01 c.c.	Lived	
2. " " "	1350	0.125 "	0.01 "	"	
3. " " "	1600	0.25 "	0.01 "	"	
4. Subcutaneously "	1440	0.25 "	0.01 "	"	
5. On opposite sides "	1770	0.1 "	0.01 "	"	
6. " " " "	1630	0.1 "	0.01 "	"	
Controls :					
1. Rabbits injected with culture only	1750		0.001 "	Died in 4 days.	Strept. infection.
2. " " " "	1870		0.001 "	Died in 24 hrs.	" "
3. " " " "	1820		0.01 "	Died in 4 days.	" "

The above results have been repeatedly obtained, and are absolutely conclusive that, as Marmorek and others have claimed, 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 five streptococci. First, the streptococcus given us by Marmorek, which was obtained from a case of angina complicating scarlet fever. 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 a few weeks ago, 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 in the horse by the streptococcus from England had nearly the same value. Against the latter two streptococci, as well as against a pneumococcus, which in ordinary cultures looks like a streptococcus, the serum had no effect.

The results published by others must also be taken to prove that a serum which protects from infection with one streptococcus may fail against others; but, taking all together, they indicate that the majority of streptococci met with in practice will be influenced by the same serum. Many more streptococci, however, must be obtained from human infections and tested before we can be certain of this. Those obtained from

human sepsis, which are not very virulent in animals, are especially in need of investigation. If those who use the serum will send to the laboratories materials for cultures this can in time be fully determined.

The Preparation of the Serum. Antistreptococcus serum is obtained from the horse, ass, and sheep after treatment by repeated injections of living streptococcus cultures. 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.

The Stability of the Serum. Unfortunately, after several weeks or months, the serum, as a rule, at least, loses its protective value. It should be kept in a cold and dark place. Not only ourselves, but others, such as Aronson, have found this to be true.

To this deterioration can probably be ascribed the failure of Koch, Petruschky and others to find in the serum any power to protect animals from infection.

The Standardization of the Value of the Serum. The value of the serum is measured by the amount required to protect against a multiple of a fatal dose of a very virulent streptococcus. The dose is usually a thousand times the average fatal amount of a very virulent streptococcus.

This method gives, as a rule, to those unfamiliar with bacteriology an exaggerated idea of the potency of the serum.

A thousand times the amount of a very virulent streptococcus culture required to kill an animal by producing septicæmia is still too little to kill by the streptococci injected; it is only their enormous multiplication in the animal which kills.

Double the fatal dose of a culture which kills only in a dose of 10 c.c. or over is a more severe test than a thousand times a very virulent one.

It is entirely different in case of an antitoxin which does not prevent primarily the growth of the germ, but neutralizes a chemical substance—its toxin.

Its 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, on account of no cultures having been made, we are in doubt as to the nature of the bacterial infection. Even when bacteriological examinations are made during life in cases of septicæmia, they are apt to fail to give us any information. Under Marmorek's supervision many cases have been injected; thus, even as far back as June, 1895, when his last statistics were published, he had treated 96 cases of scarlet fever, 411 cases of erysipelas, 16 cases of puerperal fever, and smaller numbers of cases of tonsillitis and of post-operative septicæmia.

Since then he has treated many forms of phthisis. In all these cases marked improvement is reported to have followed when they were due to streptococci. Thus, in sixteen cases of puerperal fever seven were due to streptococcus alone. All these recovered. Three were due to the streptococcus and colon bacillus and one to the colon bacillus alone. These four all died. In five, streptococci were associated with staphylococci. Two of these died, three recovered.

In phthisis where no cavities have as yet appeared the fever and sweats lessened and all symptoms improved. He did not state that any cases were absolutely cured. Marmorek's results are by far the best reported, and without casting any doubt upon the in-

tended honesty of his conclusions, it is my conviction that they give undoubtedly too favorable a view of the value of the serum.

In the comparatively few cases of puerperal fever, wound infection, scarlet fever, and bronchopneumonia that we have seen under the treatment the apparent results have not been uniform. Only occasionally did we see results which appeared to be distinctly due to the serum.

In a number of cases of septicæmia where chills had occurred daily for days they ceased absolutely or lessened under daily doses of 20 to 50 c.c. The temperature, though ceasing to rise to such high elevations, did not average more than one or two degrees lower than before the injections. 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. On bronchopneumonia, laryngeal diphtheria, and in phthisis we have seen absolutely no 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:

A single antistreptococcic serum protects healthy rabbits from infection from most of the streptococci obtained from human sepsis due to the streptococcus,

but not from all. Failure to do good in human infection cannot, as a rule, be attributed to the variety of streptococcus. 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 and even temporary suppression.

In suitable cases we are, I think, warranted in trying it, but we must 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 hinderance 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.

Care should be taken to get only recently tested serum, for after six weeks the best serum is almost inert; much on the market is worthless, and as it is

weak, and the testing for strength is still very crude, full doses 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 sufficient protective substance.

Bacteriological Diagnosis. Streptococci, using the name in its broadest 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 microscopical 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, according to Fehleisen, to excise a small piece of skin from the margin of the erysipelalous area in which the cocci are most numerous; this is crushed up and part of it transferred to a gelatin tube and to the melted agar in another tube. After shaking thoroughly the contents are poured out into Petri dishes. The gelatin is kept at a temperature of 20° C. At the end of two or three days numerous small colonies develop in the vicinity of the particles of skin. The agar plate is kept at 37° C. for twenty-four hours. It is usually sufficient, however, to make a streak culture on agar in a Petri dish with the crushed excised portion of skin and place this in the incubator at 37° C.

In septicæmia the culture method is always required to demonstrate the presence of streptococci, as the microscopical examination of specimens of blood is not sufficient. For this purpose from 3 to 5 c.c. of the blood should be drawn from the vein of the arm aseptically

by means of a hypodermatic needle, and each c.c. added to a tube of broth, in order to produce an adequate development of the cocci, which are found in small numbers in the bloodvessels. Petruschky is of the opinion that the cocci can best be shown in blood by animal inoculation. Having withdrawn from the patient 10 c.c. of blood by means of a hypodermatic 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 only in 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 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 perhaps the majority of cases of septicæmia give no positive 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 XXVIII.

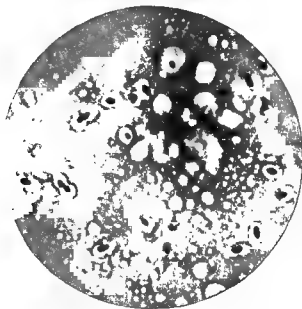
MICROCOCCUS LANCEOLATUS (PNEUMOCOCCUS ; MICROCOCCUS PNEUMONIÆ CROUPOSÆ OF STERNBERG ; MICROCOCCUS OF SPUTUM SEPTICÆMIA AND DIPLOCOCCUS OF FRAENKEL ; DIPLOCOCCUS PNEUMONIÆ OF WEICHSELBAUM).

THIS micrococcus was first observed by Sternberg, in 1880, in the blood of rabbits inoculated with his own saliva (and almost simultaneously by Pasteur under similar conditions), whence it was called by Sternberg micrococcus Pasteuri. It was subsequently described by Talamon (1883), and demonstrated by him to be capable of producing fibrinous pneumonia in rabbits when introduced into the parenchyma of the lung of these animals. In 1885 and 1886 this micro-organism was subjected to an extended series of investigations by A. Fraenkel, Sternberg, Weichselbaum, Netter and others, and proved by them to be the chief etiological factor in the production of lobar or croupous pneumonia in man.

Morphology. Very irregular; 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 individual 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.

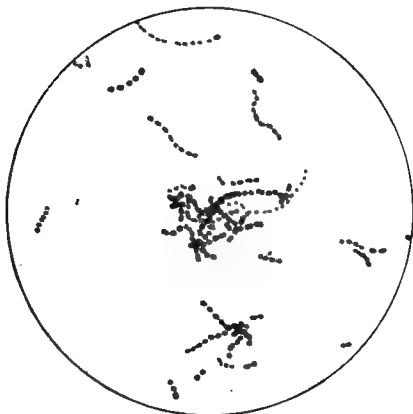
When thus united the junction, as a rule, is between the broad ends of the oval, with the pointed ends

FIG. 65.



Diplococcus of pneumonia from blood, with surrounding capsule.

FIG. 66.



Pneumococcus from bouillon culture, resembling streptococcus.

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 they are obtained. As observed in the blood of inoculated animals it is usually in pairs of lancet-shaped elements, which are surrounded by a capsule. (See Fig. 65.) When grown on culture media longer or shorter chains are frequently formed, which can scarcely be, or even not at all, distinguished from chains of streptococci. The individual cells are almost spherical in shape, and they are rarely surrounded by a capsule. (See Fig. 66.)

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.

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.

Biological Characters. It grows on almost all the culture media ordinarily employed, but its susceptibility is shown not only by its irregularity of form, but also by its slow and comparatively scanty growth and by its rapid loss of virulence and power of reproduction under varying conditions. It grows equally well in the absence as in the presence of oxygen, being thus both aërobic and facultative anaërobic; its parasitic nature is exhibited by the short range of temperature at which it grows—viz., from 25° to 42° C.—its maximum growth being at about 37° C., or the temperature of the body. Its thermal death-point, as determined by Sternberg, is 52° C., the time of exposure being ten minutes. It loses its vitality in cultures in a comparatively short time, and is very sensitive to the

action of germicidal agents. Its pathogenic power also undergoes attenuation very rapidly when cultivated on artificial media. It is non-motile.

In the cultivation of this organism one of the most important considerations is the reaction of the media employed. According to Fraenkel, Sternberg and others, it grows only in culture media when they have a slightly alkaline reaction. Kruse and Pansini showed by their investigations that, according to the source from which it was obtained, it grows at times equally well in a slightly alkaline or slightly acid medium. Not infrequently, however, all experimenters have found that no growth at all occurs, irrespective of the composition or reaction of the media employed. The weight of opinion, nevertheless, seems to be in favor of the selection of a slightly alkaline medium.

The organism grows, as has been said, on all the culture media ordinarily employed for the cultivation of bacteria—viz., on agar and gelatin, in bouillon, ascitic fluid, and blood-serum. The best medium for its growth is a mixture of one-third ascitic or pleuritic fluid and two-thirds bouillon. It grows readily in milk, causing coagulation with the production of acid, though this is not constant.

Growth on Agar. Cultivated on plain nutrient agar, after forty-eight hours in the incubator, there appears a thin, colorless layer composed of dilute non-confluent colonies. 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 resemble those of some of the streptococci growing in short chains; they are almost circular in shape, finely

granular in structure, and have a somewhat darker, more compact centre, surrounded by a paler marginal zone. With high magnification rows of cocci are seen sprouting from the edges. In stick cultures along the line of puncture minute transparent drops appear.

Growth on Gelatin. The growth on gelatin is slow, if there is any development at all, owing probably to the low temperature—viz., 22° to 24° C.—at which the gelatin has to be kept. The gelatin is not liquefied.

Growth on Blood-serum. The growth on Löffler's blood-serum mixture is very similar to that on agar, but somewhat more vigorous, 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, due to the development of the micrococci, which on microscopical examination can be seen to be arranged in pairs or longer and shorter chains. After two or three days the medium becomes again transparent, owing to the subsidence of the cocci to the bottom of the tube.

Special Media. Fraenkel was the first to draw attention to the fact that this organism soon loses its reproductive power when grown on ordinary culture media, and more particularly solid media. In fluid media the vitality is not quite so quickly lost; but even here it is found advisable in practice to transplant fresh cultures every day. By this method, when bouillon cultures are used, the vitality may be indefinitely prolonged; but after transplantation through several generations it is found that the cultures begin to lose in virulence, which finally disappears entirely. In order

to restore this virulence, or to keep it from becoming attenuated, it is necessary, therefore, to interrupt the transplantation and pass the organism through the bodies of susceptible animals.

With the view of overcoming some of 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; 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 months. Lambert has found cultures in this medium alive and fully virulent after eight months.

Löffler's blood-serum mixture is probably the best 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.

Effects of Germicidal Agents, Light and Drying. The following are the effects of germicidal and antiseptic agents on this organism, according to observations made by Sternberg: *Boric acid*, saturated solution, failed to destroy the vitality after two hours, but a solution of 1 : 400 restrained its development; *carbolic acid*, 1 per cent. solution, destroys the vitality in two hours, and 1 : 500 restrains development; *mercuric chloride*, 1 : 20,000, destroys vitality in two hours, 1 : 40,000 restrains development; *salicylic acid* and *sodium biborate*, 1 : 400 solution, restrained development.

As to its duration of life outside the body, the researches of Bordoni-Uffreduzzi throw some light. He found that pneumonic sputum attached to clothes, when dried in the air and exposed to diffuse daylight, retained its virulence, as shown by injection in rabbits, for a period of nineteen to fifty-five days. Exposed to direct sunlight the same material retained its virulence after twelve 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 albuminous material in which the micrococci were embedded. Thus, 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 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.

The Source of Infection. Although, as we have just seen, the pneumococcus may retain its virulence in dried sputa for considerable lengths of time, still such pneumococci are not the only source of contagion, for in the throat secretions of many healthy persons, and in the bronchial and lung discharges of nearly all cases of chronic pulmonary diseases, we have the pneumococci abundantly present.

Pathogenesis. The micrococcus lanceolatus is quite pathogenic for some animals—viz., mice and rabbits—less so for others. In mice and rabbits the subcutaneous injection of small quantities of pneumonic sputum in the early stages of the disease, or of a pure, virulent

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 most marked pathological lesion is the enlargement of the spleen, which in mice is conspicuous and common, and in rabbits not so much so. It is sometimes hard, dark colored, and dry, or it may be soft and bright red. The liver also is sometimes dark colored and gorged with blood, but more frequently it is paler than normal and rich in fat. The blood of inoculated animals immediately after death often contains the micrococci in very large numbers. For microscopical examination they may be obtained from the blood of the veins, arteries, or cavities of the heart, and usually from the pleural and peritoneal exudations when they are present.

Mice and rabbits are the most susceptible animals, and are thus usually employed for experimental purposes in investigations with this micrococcus; but guinea-pigs, dogs, cats, rats, and sheep are also susceptible. Chickens and pigeons are insusceptible. Young animals, as a rule, are more easily affected than old ones. In dogs subcutaneous injections usually give negative results. 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 fibrous 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.

Attenuation of Virulence. The pathological changes above mentioned apply only to the effects produced by fully virulent cultures on susceptible animals. With attenuation of virulence in the cultures or decrease of susceptibility in the animals different effects are produced. When the disease takes a rapid course the local reaction and the changes in the internal organs are comparatively slight; but the longer the process lasts the greater will be the local reaction and pathological lesions in the body. Attenuation of virulence may be produced in various ways. The loss of virulence which occurs when the micrococcus is transplanted in cultures through several generations has already been referred to. A similar attenuation of virulence takes place also spontaneously in the course of pneumonia. Patella has shown by daily puncture of the lung in different stages of the pneumonic process that the virulence of the organism diminished as the disease progressed, and that the nearer the crisis was approached the more attenuated it became—a fact which has been confirmed by others. Welch found that the most virulent micrococci were contained in the freshly hepatized portions of the lung. Fraenkel and Weichselbaum showed that the cocci taken from the lung varied in virulence according to the stage of the disease when they were obtained. Attenuation of

virulence may also be effected artificially. Banti found that the continued passage through the bodies of guinea-pigs, which are not particularly susceptible, also resulted in a loss of virulence. Sanarelli states that the cultivation in human saliva is also attended with an attenuation of virulence. Cultivation in other unfavorable media, or media to which substances have been added which restrain development, has similar attenuating effect on the virulence.

Restoration and Increase of Virulence. The simplest and perhaps the most reliable method of restoring lost virulence for any animal is by passage through the bodies of highly susceptible animals of the same species.

Occurrence in Man. The micrococcus lanceolatus is not infrequently present in the saliva of healthy individuals, having been found by Sternberg in the oral cavity of about 20 per cent. of healthy persons examined. It is constantly to be detected in the rusty sputum of patients suffering from acute fibrinous pneumonia. Weichselbaum reports having found it in 94 out of 129 cases of pneumonia examined by him; Wolff found it in 65 cases out of 70 examined; Netter in 75 per cent. of his cases, and in the sputum of convalescents from pneumonia in 60 per cent. The more recent the infection the greater is the number of bacteria found in the diseased lung areas. As the disease progresses they 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. But lobar pneumonia is not the only form of pneumonia in the production of which this organism is concerned. 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 micrococcus lanceolatus. The microscopical appearances in bronchopneumonia are the same as in lobar pneumonia, the only difference being, according to the observations of Ribbert and Baumgarten, that in the former the infective process is less extended, resulting in the formation of a number of small foci instead of the lung being attacked *in toto*.

Beside the affections above mentioned, this micrococcus is associated with other pathogenic bacteria, producing a secondary or mixed infection. In tuberculosis, for example, it is often found associated with the tubercle bacillus, taking part with this organism in the destruction of the tubercular tissue of the lungs. Having once reached the lung it may penetrate to different organs in the body, producing in them more or less intense inflammatory processes, which are mostly of a purulent character. It may thus cause inflammations of the serous membranes of the endocardium, the pericardium, the meninges, and even of the brain itself. Foremost among the secondary infections which it causes are meningitis, serofibrinous pleurisy, and empyema. In 25 cases of purulent meningitis examined by Netter the "pneumococcus" was found in 16; 4 of these cases were complicated with purulent otitis, 6 with pneumonia, and 3 with ulcerative endocarditis. In 45 cases collected by Netter from the literature of

the subject this micrococcus was present in 27. Monti demonstrated the presence of the same micrococcus in 4 cases of cerebro-spinal meningitis. Weichselbaum, in a series of 29 cases of ulcerative endocarditis examined, found "*diplococcus pneumoniae*" in 7. It has been found also in acute abscesses—in the pus of parotitis complicating pneumonia it was obtained in pure culture by Testi; in a case of pneumonia, in which there developed a purulent pleuritis and parotitis, and multiple subcutaneous abscesses, it was found in great numbers in all these places in the pus; in a case of tonsillitis resulting in the formation of an abscess it was obtained in pure culture by Gabbi. This micrococcus has also been found in a number of cases of otitis media—in the pus obtained by paracentesis of the tympanic membrane, by Netter in 5 out of 18 cases occurring in children. Monti and Belfanti report cases of arthritis of the wrist-joint, occurring as a complication of pneumonia, in which it was found. Ortmann and Samter, in a case of purulent inflammation of the shoulder-joint following pneumonia and pleurisy, obtained the "*pneumococcus*" in pure culture. It has been found in other cases of inflammation of the knee, ankle, and elbow-joints, and in osteomyelitis and periostitis. In short, there is scarcely any part of the body in which this organism may not find suitable conditions for existence and in which it does not sometimes occur.

How is it conveyed from its original seat in the lungs to distant internal organs? Chiefly by means of the bloodvessels 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 chock-full of 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 intra-uterine infection in pneumonia and meningitis. These investigators have demonstrated the presence of the micrococcus lanceolatus in foetal 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 pneumonia which are caused by this micrococcus; and not only the secondary, but also the primary diseases, as of the brain and the 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, excesses of various kinds, etc., to readily comprehend how an individual may become infected with pneumonia, 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.

From statistics collected by Netter the following percentages of diseases were caused by the "diplococcus pneumoniae":

Pneumonia	65.9 per cent. in adults.
Bronchopneumonia	15.8 " "
Meningitis	13.0 " "
Empyema	8.5 " "
Otitis media	2.4 " "
Endocarditis	1.2 " "

In 46 consecutive pneumococcus infections in children there were :

Otitis media	29 cases.
Bronchopneumonia	12 "
Meningitis	2 "
Pneumonia	1 "
Pleurisy	1 "
Pericarditis	1 "

Varieties of the Micrococcus Lanceolatus. The ubiquity of this organism and the irregularity of its behavior under varying conditions have opened a wide field of discussion among bacteriologists. As commonly found, for instance, in the saliva of different healthy individuals, and even in that of the same individual at different times, it often varies in virulence;

and as obtained from the saliva it differs again from the organism when occurring in pneumonia. When grown on artificial culture media, variations in its morphology have been observed, at one time the individual cells being oval in shape and united in pairs, and then surrounded by a capsule; at other times spherical and arranged in longer or shorter chains, like streptococci, and then being without a capsule. Variations in virulence have also been noted, both in the animal body in different stages of disease and when grown outside the body on artificial culture media. These great variations in biological and pathogenic properties have induced some investigators to believe that there were several distinct species of this organism.

These views, however, have not met with general acceptance. In an exhaustive investigation into the subject by Kruse and Pansini, who obtained the micrococci from the most varied sources—from the saliva in health and disease, from the nasal secretions, from pneumonic sputum at different periods and phases of the illness, from the blood of different kinds of animals killed by inoculation, and, finally, from many primary and secondary affections due to this organism—after carefully weighing their results from different points of view and comparing the morphological, biological, and pathological characteristics of the various micrococci found, these observers have come to the conclusion that “it is impossible to distinguish different varieties” of pneumococci. They found numerous quantitative and qualitative variations in virulence, growth, power of resistance, etc., but at the same time such an inconstancy in these variations that they were unable to make any classification into separate varieties.

Judging from the streptococcus, the use of a specific bactericidal serum developed from a single pneumococcus will probably show that some of the organisms ranked as pneumococci are not influenced by it.

Immunity. Early in the history of this organism experiments were begun for the production of immunity in animals by means of preventive inoculations. Fraenkel showed that subcutaneous injections of rabbits with virulent cultures of the diplococcus produced infection in only a small percentage of these animals, which either died from septicæmia or after a time recovered. In the latter case they were found to be somewhat immune to a second infection. Later experiments were conducted on the same principle, the object being to repeatedly slightly infect the animal, and thus to gradually increase its power of resistance to infection. For this purpose either artificially attenuated cultures or material containing naturally attenuated micrococci were used for inoculation. Cultures artificially attenuated by heat or several days' growth in the incubator, sputum taken from a pneumonic patient after the crisis, rusty sputum obtained before the crisis and heated to 60° C., old pleuritic exudation containing attenuated bacteria, etc., have thus been repeatedly employed.

Another series of experiments were based on the assumption that the immunizing substances are contained in the natural or artificial products of the growth of the organism. Thus cultures which were freed from bacteria by filtration, and emulsions of pneumonic sputum, portions of pneumonic lung, pleuritic exudations, etc., were employed by different experimenters. The quantity of material required for inoculation being found inconveniently large, attempts were then made

to obtain the immunizing substances in a more concentrated form. Foá and Scabia and F. and G. Klemperer prepared glycerin extracts, after the manner of Koch, calling their extract "pneumoprotein." At present, however, a protective serum is obtained from horses by the repeated injections of fully virulent pneumococci in exactly the same manner as in the production of antistreptococcus serum.

Therapeutic Experiments. Curative experiments in man have also recently been made with the blood-serum of immunized animals and of persons who have recovered from an attack of pneumonia. The most successful of these were conducted by F. and G. Klemperer. These authors hold that in man during the pneumonic process there is a constant absorption into the circulation of this toxic albuminous substance produced by the bacteria in the lungs. This continues until eventually the same antitoxic substance is produced in the circulation that has been seen to occur experimentally. It is then that the crisis occurs. The bacteria are neither destroyed nor is their power to produce pneumotoxin lessened; but the third factor—the antipneumotoxin—now exists and neutralizes the toxic substances as they are produced. They apparently demonstrated that the serum of the blood of patients after the crisis of pneumonia contained antitoxic substance, and was capable, in a fair number of cases, of curing the disease when injected into infected animals. They have made preliminary observations upon patients with a view of inducing the crisis by the injection of the blood-serum of persons convalescent from pneumonia, and which, consequently, contain the antitoxic body. In six pneumonic patients the results were promising.

In all there was a decided fall of temperature in from six to twelve hours after subcutaneous injections of from 4 to 6 c.c. of the serum. The pulse and respirations were also diminished in frequency. In two cases the temperature fell to 37° C. Twice it fell and remained at normal. In other cases it fell only temporarily.

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, although considerable, is still too few to warrant the expression of any 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 antipneumotoxic serum are practically harmless.

CHAPTER XXIX.

DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS.

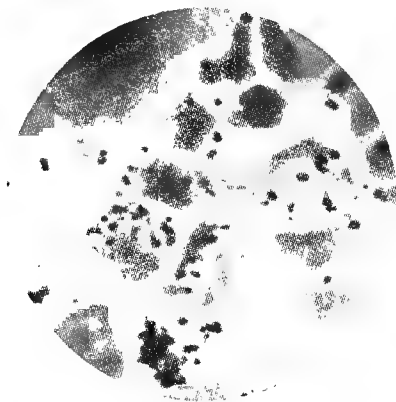
IN the description of the micrococcus lanceolatus reference was made to this organism as the most frequent cause of meningitis, especially when it complicated pneumonia. In 1887, Weichselbaum discovered another micrococcus in the exudate of cerebro-spinal meningitis in six cases, two of which were not complicated by pneumonia. He obtained it in pure cultures, studied its characteristics, and showed that this organism was clearly distinguishable from the micrococcus lanceolatus, and especially by its usual presence in the interior of pus-cells, on which account he called it *diplococcus intracellularis meningitidis*. The frequency of the occurrence of this diplococcus in meningitis and its restriction to this disease affords sufficient evidence for the assumption that it was concerned in its production. In 1895, Jaeger and Scheurer found it in the nasal secretions of eighteen living persons suffering from this disease during an epidemic.

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 smaller degenerated forms are found. In the exudation, like the gonococcus, to which it bears a close resemblance in form and arrangement, it is distinguished by its presence within the polynuclear leucocytes. It

never appears within the nucleus and rarely within other cells (Fig. 67).

It *stains* with all the ordinary aniline colors, but best with Löffler's methylene-blue. According to Weichselbaum, it is decolorized by Gram's solution; Jaeger states that this is not constantly the case.

FIG. 67.



Diplococcus intracellularis meningitidis. $\times 1100$ diameters.

Biological Characters. It does not grow at room-temperature, but only in the incubator, and its development is usually scanty on the surface of agar, but sometimes a few colonies grow quite vigorously. It does not grow at all or scarcely any in bouillon, and very scantily in bouillon plus one-third blood-serum. It grows comparatively well on Löffler's blood-serum medium as used for diphtheria cultures.

When grown on nutrient agar or glycerin-agar, at the end of forty-eight hours in the incubator, there develops a tolerably good growth, appearing as a flat layer of colonies, about one-eighth of an inch in diam-

eter, grayish-white in color, viscid and non-confluent unless very close together. On Löffler's blood-serum the growth forms round, whitish, shining viscid-looking colonies, with smooth and sharply-defined outlines, and 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. 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 as finely granular colonies, 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. Cultivated in artificial media it soon loses its vitality—within six days—and requires, therefore, to be transplanted to fresh material at short intervals—at least every two days.

Pathogenesis. This organism does not show much pathogenic power for animals. It is most pathogenic for mice and guinea-pigs, less so for rabbits and dogs. Subcutaneous injections in animals give negative results; intrapleural or intraperitoneal inoculations in mice and guinea-pigs, when given in large doses, are usually successful. Intravenous injections in rabbits have caused the death of the animal, but no diplococci or pathological changes have been found as a result of the injections.

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 fibrino-purulent exudation. In the blood and enlarged spleen

diplococci are found in small numbers and mostly free; in the pleuritic exudation they are present in considerable quantities, less so in the peritoneal fluid, but then occurring in the interior of pus-cells.

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, between the dura mater and the brain, at the point of inoculation, was found a thick, reddish, purulent liquid; in the brain itself an abscess had formed, about the size of a hazelnut, filled with tough, yellow pus, while the abscess walls consisted of softened brain-substance infiltrated with numerous hemorrhagic deposits, and simultaneously 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. Weichselbaum suggests that under natural conditions the diplococci gain access to the brain and meninges by way of the nose, ear, and upper air-passages. Cerebrospinal meningitis, as is well known, is often accom-

panied by rhinitis and purulent inflammation of the mucous membranes of the nose. In one of his six cases Weichselbaum succeeded in obtaining in pure culture diplococci from the nasal secretion. Scheurer, in his eighteen cases, found the diplococci in the nasal secretions of all of them during life. In fifty healthy individuals examined they were found in the nasal secretions of only two of them, one being a man suffering at the time from a severe cold. This man, it is interesting to note, had been engaged in disinfecting a room which had previously been occupied by a patient with cerebro-spinal meningitis.

Bacteriological Diagnosis. By means of lumbar puncture fluid can be readily obtained without danger from the spinal canal. The microscopical examination will frequently reveal numerous cells crowded with diplococci. When considerable quantities are inoculated upon Löffler's blood-serum mixture or upon glycerin agar, 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 40 per cent. of the cases due to this coccus recover, while almost all of those due to the pneumococcus and streptococcus die. In fifty-five cases examined by Councilman, Mallory, and Wright, diplococci were found in the fluid removed by lumbar puncture in thirty-eight, either by microscopical examination or cultures.

The longest time after the onset of the disease in which positive results were obtained by culture was twenty-nine days. In a number of cases examined by us for Northrup a rather smaller percentage of the cases were found to be due to this diplococcus. In many cases

there are very few diplococci present in the spinal fluid, so that a failure to find them in a microscopical examination should not be taken to prove that the disease was not due to this organism. 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 lumbar vertebræ. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is entered about 1 cm. to the right of the median line 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 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 microscopical 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.

CHAPTER XXX.

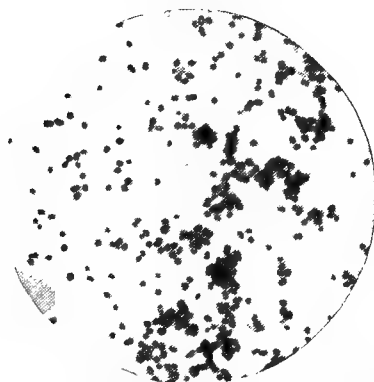
MICROCOCCUS GONORRHŒÆ (GONOCOCCUS NEISSER).

THIS micrococcus was first observed by Neisser, in 1879, 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 and isolate it and then prove its infective virulence by inoculation into man. Since that time the gonococcus has been cultivated on various media, which, though modifications of Bumm's, are an improvement on his original method, and as the result of various inoculation experiments there now remains no doubt that this organism is the specific cause of gonorrhœa in man.

Morphological Characters. Micrococci, occurring mostly in the form of diplococci—that is, in pairs or in groups of four. 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 gives them their characteristic "coffee-bean" or "biscuit" shape (Fig. 68). The diameter of an associated pair of cells varies from 0.8μ to 1.6μ in the long diameter—average about 1.25μ —by 0.6μ to 0.8μ in the cross diameter. In gonorrhœa gonococci are found mostly in small, irregular groups in or upon the pus-cells, and

generally extranuclear. When found in other portions of the field this is mostly due to the mechanical effect of smearing the pus on cover-glass slides, and should not be considered as characteristic. That the gonococci really lie within the protoplasm of the cells is proved by the fact that in carefully made preparations they are usually not found outside of the pus-cells. They appear usually as diplococci, in groups of two or four,

FIG. 68.



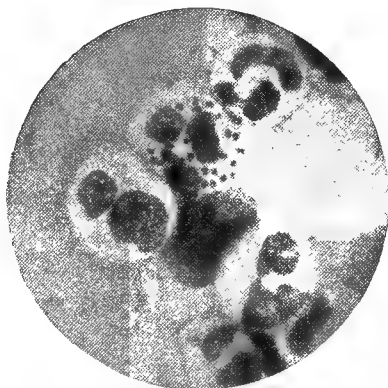
Smear from pure culture of gonococcus on agar. (HEIMAN.)

but at times they occur as round, single, and undivided cells. Others, again, are irregular in shape or granular in appearance, involution forms, particularly in older cultures and in chronic urethritis of long standing. The pus-cells containing gonococci are most numerous in the later or purulent stage of the disease, not so frequent in the beginning of infection, or as long as the discharge is of a serous character (Fig. 69).

The gonococcus *stains* readily with the basic aniline colors, especially with methyl-violet, gentian-violet, and

fuchsin; not so readily with methylene-blue, which is, however, one of the best staining agents for demonstrating its presence in pus. Beautiful double-stained preparations may be made from gonorrhœal pus by treating cover-glass smears with methyl-violet and eosin. Gonococci are decolorized by Gram's solution; but this cannot be depended upon alone to absolutely distinguish the gonococcus from all other diplococci

FIG. 69.



Gonococcus in pus-cells. $\times 1100$ diameters.

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.

Biological Characters. The elaborate experiments of Bumm and others have shown that at the ordinary room-temperature no growth of the specific micrococcus occurs on the culture media. Apparently positive results which have been reported are found to be due to other diplococci morphologically almost identical with the gonococcus.

Since Bumm's experiments a number of culture methods have been proposed for the gonococcus which are an improvement on Bumm's, partly because the growth produced is more constant and luxuriant and partly because the media employed are more readily prepared. Wertheim (1892) succeeded in developing luxuriant and virulent cultures to many generations on a mixture of placenta blood-serum and 2 per cent. peptone-agar. His method is briefly as follows: Several loops of gonorrhœal pus are diffused through liquid blood-serum warmed to 40° C. contained in a test-tube. Two dilutions are made from this, and an equal quantity of melted 2 per cent. agar cooled to 40° C. is added to the three tubes, and the contents, after thorough mixing, poured into Petri dishes. The Petri dishes are placed in an incubating oven at a temperature of 36° to 37° C. At the end of twenty-four hours there will have developed on at least one of the plates distinct colonies; these are translucent, finely granular, with scalloped margin. By transferring such a colony to slant-cultures of serum-agar, pure cultures of the gonococcus are obtained; these are somewhat shining in appearance and of a grayish-white color.

Wertheim demonstrated that the addition of peptone to the culture medium was an important factor in the cultivation of gonococci. Kiefer (1895) proposed a culture medium consisting of one part of hydrothorax or ascitic fluid and one part of a fluid containing 3.5 per cent. agar, 5 per cent. peptone, 2 per cent. glycerin, and 0.5 per cent. salt.

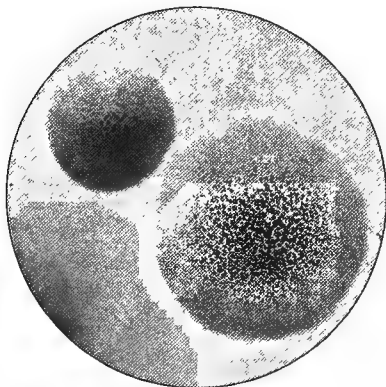
Simultaneously with Kiefer, Heiman recommended a medium made from hydrocele fluid, or from "chest-serum" obtained from a patient suffering from hydrocele or hydrothorax or acute pleurisy. Having experimented with all the various culture media heretofore prepared for the cultivation of the gonococcus, Heiman believes this medium to be superior to placenta-serum, sheep blood-serum, or ascitic fluid, because of the large amount of serum albumin which it contains. The medium consists of a 2 per cent. agar, plus 2 per cent. peptone, plus 0.5 per cent. salt and 2 per cent. glucose. Of this mixture two parts are added to one part of chest-serum, which is, if necessary, fractionally sterilized between 65° and 70° C. for one hour for seven consecutive days. The chest-serum-agar should have a neutral reaction. The growth on this medium is thus described: In plate cultures streaked on the surface, growth abundant, colonies circular in shape, edges somewhat irregular, shading off into yellowish-white; texture finely granular in periphery, presenting punctated spots of higher refraction in and around the centre of yellowish color (Fig. 70).

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). No development takes place below 25° C. or above 39° C.; it is killed at a temperature over 42° C.

Pathogenesis. Non-transmissible to dogs, monkeys, horses, and rabbits, whether inoculations be made into the urethral, vaginal, or congenital mucous membranes. According to Wertheim, purulent peritonitis, not caus-

FIG. 70.



Colonies of gonococci on pleuritic fluid agar. (HEIMAN.)

ing death, is produced in certain animals by the introduction of pieces of serum-agar containing colonies of the gonococcus. This effect was produced constantly in mice, occasionally in guinea-pigs, and rarely, if ever, in dogs, rats, and rabbits.

Though animal inoculations are thus followed by negative results, the etiological relation of the gonococcus to human gonorrhœa has been demonstrated beyond question by the infection of healthy men with the disease by inoculation. Thus, Bumm has produced

gonorrhœa in normal urethral mucous membranes by inoculation of a pure culture on blood-serum in the second generation; Wertheim, in the thirtieth; Kiefer, in the sixth, and Heiman in the fifth generation. At the same time the distinctive morphological, staining and biological characters of the organism were carefully noted and confirmed to be those of the gonococcus by these observers; the typical incubation and symptoms of the disease resulted in all cases in the subjects experimented on.

According to the observations of the most reliable investigators and those most familiar with the various forms of micrococci which are likely to be mistaken for the gonococcus, affections due to this organism are usually restricted to the mucous membranes of the urethra, conjunctiva, bladder, cervix uteri, and rectum. It rarely, if ever, produces a vaginitis in adults; but occasionally a vulvo-vaginitis in young children. Formerly the presence of gonococci could only be determined microscopically; but since the introduction of the serum-agar the culture method has rendered the diagnosis of gonorrhœa much more reliable. This method of investigation, moreover, has given valuable information with regard to the nature of many infections complicating or resulting from gonorrhœa, particularly in affections of the uterus and joints, about which there was heretofore considerable doubt, though the micrococci often found in these organs were morphologically identical with the gonococcus. It has now been shown by the culture method that gonococci may occur in the joints in gonorrhœal arthritis, in the Fallopian tubes in salpingitis, and in ovarian abscesses; and Wertheim asserts that he has found them in the infiltrated connective tissue in parametritis.

Worthy of special notice in this connection are the cases of endocarditis accompanying gonorrhœa, and sometimes terminating fatally, when they are known as *endocarditis gonorrhœica maligna*. The question naturally arises in these cases whether it is the gonococcus or some other coccus or diplococcus which has infected the endocardium. Here, again, it is only by means of the culture method that this question can be settled definitely. Flügge draws attention to this matter, and states that but few cases have been recorded in which the information given as to the cause of the disease can be unhesitatingly accepted. Weichselbaum mentions a case of endocarditis accompanying gonorrhœa which was shown by the culture method to be due to streptococcus infection, proving that so-called gonorrhœal endocarditis may be a secondary infection. Other cases are recorded by Leyden, Hiss, Councilman, and Wilms which are said to have been *most probably* of gonorrhœal origin; but in these only microscopical examinations were made and no culture experiments, or only cultures on gelatin plates, etc., which were inadequate. Welch also reports a case of endocarditis with general septicæmia following gonorrhœa, in which he demonstrated the gonococcus in the blood of a living person in cover-glass and culture medium. No other pathogenic bacteria were found.

Immunizing Serum. As animals are not infected by the gonococcus they are not very suitable for injections with the cultures for the purpose of producing an anti-toxic or bactericidal serum. Their insusceptibility presents also an almost insurmountable obstacle to the testing of the blood of animals under treatment, so that although it may be possible to bring about an artificial

immunity against gonorrhœal infection, information on this subject is at present wanting. Immunity in man seems to be similar to that produced after infection with the other pyogenic cocci—that is, only slight in amount and for a short period. It is known that a urethra or cervix 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 the one carrying the infection or in another person.

The Bacteriological Diagnosis of Gonorrhœa. In view of the fact that several non-specific forms of urethritis exist, and also that micrococci morphologically similar to the gonococcus Neisser are often found in the normal urethral and vulvo-vaginal tract, it becomes a matter of great importance to be able to detect gonococci when present and to differentiate these from the non-specific organisms. The gonococci also which occur in old cultures and in chronic urethritis of long standing often take on a very diversified appearance—sometimes nothing but an irregular, granular mass being seen, which renders their detection difficult. From a medico-legal and social stand-point, 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 microscopical and the cultural. Animal inoculations are of little value, as they are not susceptible, and, of course, human inoculations are, except in extremely important cases, generally impossible. In the microscopical diagnosis it should be borne in mind that the specific gonococci in *carefully made* preparations are found always largely within the pus-cells. Diplococci morphologically similar to gonococci occur-

ring in other portions of the field and outside of the pus-cells should not be considered specific by this 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 vulvo-vaginal tract the certainty is not so great; here cultures should also be made. Bumm, Heiman and others have shown that other diplococci are occasionally found in gonorrhœal pus from the vulvo-vaginal tract, and very rarely, indeed, from the urethra, which do not stain by this method. Cover-glass preparations from subacute or chronic cases should be examined, if possible, with a microscope provided with a mechanical stage, and should always be stained 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 on chest-serum-agar, poured in dishes, as proposed by Heiman, also, if with negative results, on three consecutive days. His method of procuring the urine in chronic urethritis is to allow 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 slightly turbid at the bottom of the tube. This turbidity will be found, on microscopical examination, to consist of epithelial cells, a few leucocytes, and some bacteria.

Heiman looks upon the decolorization by Gram's method as the only reliable criterion, so far as known, for the gonococcus in discharges from the mucous membranes, and it is of material help, also, in determining whether a culture is or is not that of the gonococcus. The careful examination of gonorrhoeal threads with cover-glass 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 proved. 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. 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. Heiman was able to confirm the correctness of the above allusion, for on one occasion, in examining threads, when he could not demonstrate the gonococcus in cover-glass preparations, he succeeded in growing it on chest-serum-agar plates, while in all instances in which he found the gonococcus in threads in cover-glass preparations he invariably succeeded in growing it on chest-serum-agar plates. The culture methods, of course, presuppose that one has the facilities and knowledge to carry them out successfully, otherwise the microscopical methods are to be used alone.

In acute cases 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. This is a far less satisfactory method than that suggested by Heiman, by "centrifuging," except when the pus is abundant.

The Frequency with which Gonococci are Found in Smears or Cultures in Cases of Chronic Urethritis. Heiman found in 61 cases 14 by cultures and 13 by smears. The following results were obtained by other observers by cover-glass preparations: Goll, according to his elaborate article, 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. Weinrich, out of 25 similar cases, obtained 2 positive results. E. Noeggerath, in 1887, deplored the fact that on account of the lack of culture media for the gonococcus we cannot always demonstrate them.

Bröse, in 1893, stated that the culture medium is the only reliable agent for the detection of the gonococcus. This latter statement is certainly applicable to chronic urethritis of the male. Neisser, in 1893, stated that in chronic urethritis with slight discharge the examination with a culture medium for gonococci will replace the cover-glass.

CHAPTER XXXI.

BACILLUS PYOCYANEUS (BACILLUS OF GREEN AND OF BLUE PUS)—BACILLUS PROTEUS VULGARIS—BACILLUS OF MALIGNANT OEDEMA—BACILLUS AËROGENES CAPSULATUS.

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.

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.

Biological Characters. An 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 with coincident acid reaction.

• There is some difference of opinion with regard to the pigments produced by the bacillus pyocyaneus. Gessard's view is that two pigments are produced by this bacillus—one of a fluorescent green and the other (pyocyanin) of a blue color. Pyocyanin is soluble in chloroform, and may be obtained from pure solution in long, blue needles. This pigment, which is thus extracted by chloroform, distinguishes the bacillus pyocyaneus from other fluorescing bacteria.

Pathogenesis. This bacillus is very widely distributed in nature; it is found on the healthy skin of man, in purulent discharges and in serous wound secretions. Its presence in wounds greatly delays the process of repair and may give rise to a general depression of the vital powers from the absorption of

its toxic products. Its pathogenic effects on animals have been carefully studied. It is pathogenic for guinea-pigs and rabbits. Subcutaneous or intra-peritoneal injections of not too small quantities of a recent culture—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. Immunity may also be secured by the injection of a considerable amount of a sterilized culture. 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. Similar results have been obtained by Woodhead and Wood by the injection of sterilized cultures of this bacillus, made immediately after injection with the anthrax bacillus. 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 slight protecting value in the body.

Our knowledge of the pathogenic importance of the bacillus pyocyaneus in human diseases has been much increased by recent investigations. Thus cases have been reported in which 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 same micro-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 pyocyaneus in serous inflammation 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 turn out 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. Jadkewitsch reports the case of a patient suffering from eczema of the lower extremities, in whom three times during a period of ten years there was eruption of boils containing blue pus, with accompanying symptoms of poisoning, emaciation, prostration, diarrhœa, and paresis. Krambals refers to seven cases in which a general pyocyaneous 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. Schimmelbush 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. Neumann has obtained the bacillus pyocyaneus in pure culture in two cases of hæmatemesis and melæna of the new-born from the blood and other organs. 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.

We may, therefore, conclude from these facts that the bacillus pyocyaneus, although ordinarily non-pathogenic for man, may under certain conditions become a dangerous source of infection. Children would seem to be particularly susceptible to this infection.

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.

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.

Biological Characters. 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.

The 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 presence 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, into 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 that 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 feces of healthy infants. "The prominent symptoms in the cases of cholera infantum in which the proteus bacteria were found were drowsiness, stupor, emaciation, 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 micro-organism 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, however, 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. On the whole, considering the very wide distribution of this organism in nature, it is remarkable how few diseases are produced by it.

BACILLUS OF MALIGNANT ŒDEMA.

This bacillus is widely distributed, being found in the superficial layers of the soil, in putrefying substances, in the blood of animals which have been suffocated (by invasion from the intestine), in foul water, etc. It was discovered (1877) by Pasteur in animals after injections of putrefying liquids, and named by him "vibrion septique." He recognized its anaërobic nature, but did not obtain it in pure culture. Koch (1881) carefully studied this micro-organism, described it in detail, and gave it the name "bacillus œdematous maligni." It was isolated first in pure culture by Liborius.

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. 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 or spindle shape.

The bacilli *stain* readily by the usual aniline colors employed, but are decolorized by Gram's method.

Biological Characters. A strictly anaërobic, liquefying, motile bacillus. Forms spores. It grows, however, in all the usual culture media in the absence of oxygen. Development takes place at the room-temperature, 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. 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 a peculiar, disagreeable odor.

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. There is no odor developed, and

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 feces, 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, 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.

BACILLUS AËROGENES CAPSULATUS.

Found by Welch in the bloodvessels of a patient suffering with aortic aneurism; 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. 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.

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.

Biological Characters. An anaërobic, non-motile, non-liquefying bacillus. Does not form spores. Grows at the room-temperature, but more rapidly 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. In *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* is rapidly coagulated.

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 bloodvessels and organs, especially the liver. The following 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 into rabbits, which are killed, kept at 37° C., and cultures made twenty-four hours later from their bodies.

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 micro-organism entering the circulation and developing shortly before and after death. The bacillus has been found in the dust of hospitals.

CHAPTER XXXII.

BACILLUS ANTHRACIS—BACILLUS ANTHRACIS SYMPTOMATICI (ANTHRAX BACILLUS).

BACILLUS ANTHRACIS.

ANTHRAX is an acute infectious disease which is very prevalent among animals, particularly sheep and cattle. Geographically and zoologically it is the most wide-spread 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 pustule, and the internal anthrax, of which there are intestinal and

pulmonary forms, the latter being known as "wool-sorter's disease."

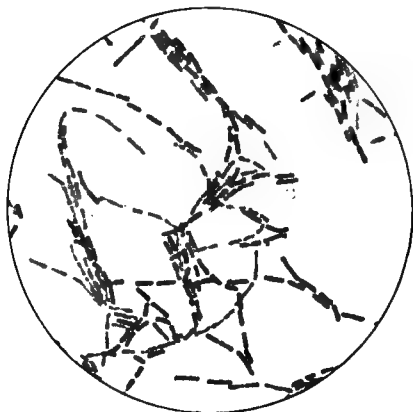
Owing to the fact that anthrax was the first infectious disease which was shown to be caused by a specific micro-organism, and to the close study which it received in consequence, this disease has probably contributed more to our general knowledge of bacteriology than any other infectious malady.

Pollender observed in 1849 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 relation of the micro-organism to the disease with which his investigation showed it to be constantly associated. For a long time this conclusion was energetically opposed until, in 1879, Pasteur, Koch 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 2 or 3μ to 20 or 25μ in length. They vary thus very much in their 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. 71 and Fig. 13, p. 47, and Fig. 17, p. 207.) 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 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

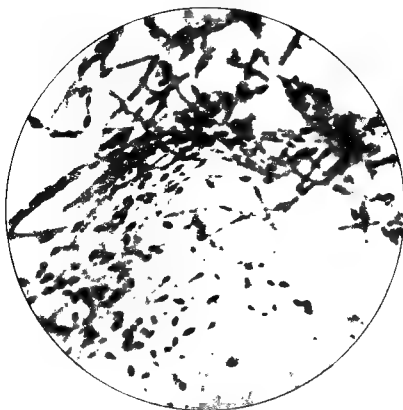
FIG. 71.

Anthrax bacillus. $\times 900$ diameters. Agar culture.

was laid upon these peculiarities as distinguishing marks of the anthrax bacillus; but it has been found that these 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 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. (Fig. 72, Fig. 13, p. 47, and Fig. 17, p. 207). Irregular sporulation sometimes takes place, and occasionally there is no spore-formation, as in varieties of non-spore bearing anthrax.

FIG. 72.



Spores heavily stained (in specimen red). Bodies of disintegrating bacilli faintly stained (in specimen blue). $\times 1000$ diameters.

The anthrax bacillus *stains* readily with all the aniline colors, and also by Gram's method, when not left too long in the decolorizing iodine 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.

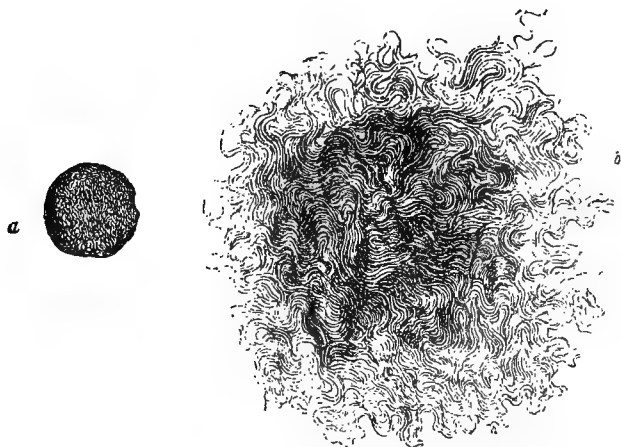
Biological Characters. The anthrax bacillus grows easily in a variety of nutrient media at a temperature from 18° to 43° C., 37° C. being the most favorable temperature. Under 12° C. no development takes place, as a rule, though by gradually accustoming the bacillus to a lower temperature it may be induced to grow under these conditions. Under 14° C. and above 43° C. spore-formation ceases. The lower limit of growth and 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 of 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.

This bacillus grows best in neutral or slightly alkaline media. It may be cultivated in infusions of meat or of various vegetables, in urine, etc., provided the

reaction be not decidedly acid, which arrests development. It grows in cow-dung and in more or less contaminated earth. 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

FIG. 73.



Colonies of bacillus anthracis upon gelatin plates. *a*, at the end of twenty-four hours; *b*, at the end of forty-eight hours. $\times 80$. (F. FLÜGGE.)

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. 73).

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 incubator 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.

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 free 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 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 unfavorable conditions. The addition of antiseptics, as carbolic acid, favors these conditions. Varieties differing in their pathogenic power may also be produced artificially. Pasteur produced an "attenu-

ated virus" by keeping his cultures for a considerable time before replanting them upon fresh soil. Chamberland and Roux have shown that cultivation in the presence of certain chemical substances added to the culture medium, as bichromate of potassium, causes an attenuation of virulence. Attenuation of pathogenic power is also effected by cultivation in the body of a non-susceptible animal, like the frog (Lubarsch, Petruschky); or in the blood of a rat (Behring); by exposure to sunlight (Arloing); to heat, 50° C. for eighteen minutes; and by compressed air (Chauveau).

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. The bacilli, in the absence of spores, are destroyed in ten minutes by a temperature of 54° C. Anthrax spores in a desiccated condition are destroyed in four hours when exposed to the action of direct sunlight, only after several weeks in diffuse daylight (Kruse).

Pathogenesis. The anthrax bacillus is pathogenic for cattle, sheep (except the Algerian race), horses, swine, mice, guinea-pigs, and rabbits. Rats, cats, dogs, chick-

ens, 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.

The anthrax bacillus produces in susceptible animals 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 cause infection. It is difficult to produce infection by the ingestion even of spores; but it may readily be caused by inhalation, particularly by the inhalation 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, 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 the capillaries will be seen to be stuffed full of bacilli, as in the glomeruli of the kidneys, 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. The anthrax bacilli in these animals seem to live almost exclusively in the bloodvessels and to leave them only by means of hemorrhages. In this way they reach—but only late in the disease—the various secretions of the body, the urine, the intestinal secretions, and occasionally the bile. The passage of the anthrax bacillus from the mother to the fœtus in pregnant females is possible, as has been shown by the investigations of Strauss, Chamberlain, and others, but it very rarely occurs.

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 destroyed by the gastric juice (Koch, Gaffky, Löffler). 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 in enormous masses.

~~NEW~~ ~~WYLL~~ 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 very rarely fatal—in only 5 per cent. of cases (Nassarow and Müller).

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 microscopical 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-sorter's 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 Gerlier, who states that of 400 persons who were 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, fol-

lowed by vomiting, diarrhœa, moderate fever, and pains in the legs and back. In acute cases there may be dyspnoea, cyanosis, and toward the end convulsions. The pathological lesions are similar to those described in animals.

Wool-sorter's disease, or pulmonic anthrax, is found in large establishments in which wool and hair are sorted and cleansed, and is 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.

Many theories have been advanced to account for the occurrence of intestinal anthrax among cattle and sheep, which in these animals is the most wide-spread form of the disease. It has been thought that infection was produced mainly by the eating of food contaminated by anthrax spores derived from the bodies of infected animals; but only in rare instances has it been possible to trace the cause of the disease to this source. The grazing of cattle on infected pastures has also been assigned as the cause of the disease; but this does not explain the occurrence of epidemics or the infection of cattle on pastures which have never before been visited by animals affected with anthrax. By some anthrax has been called a miasmatic disease and likened to

malaria. Occurring as it does at the same season of the year—viz., from June to October—and being connected apparently, like malaria, in some way with the condition of the soil, there is a certain analogy between these two diseases. Anthrax is found to occur mostly in low, swampy localities, where the soil is covered with decaying vegetable matter, and subject to overflows and freshets. There is no doubt that this bacillus is able to lead a saprophytic existence for some time, under favorable conditions, in the superficial layers of the soil, remaining latent in the form of spores and retaining its vitality; but why an epidemic of anthrax occurs one year at a certain place and at the same place the next year does not, it is not easy to explain. Pasteur believes that the earth-worm plays an important part in bringing to the surface and distributing the spores which have been propagated in the buried carcass of an infected animal; but Koch has shown that this hypothesis is both improbable and superfluous. Apart from the fact that sporulation does not normally take place inside the bodies of dead animals, the earth-worm is ill adapted for the transportation of anthrax spores, which are unfavorably affected in their intestines. Out of seventy-two earth-worms examined by Ballinger from a notoriously infected locality, only one contained anthrax spores. Furthermore, the soil in places where such carcasses lie buried is already saturated with the fluids and other products of decomposition of the body of the dead animal containing bacilli, which under suitable temperature conditions may form spores and thus infect the surface of the land; though it is possible that the earth-worm, in some instances, may contribute to the distribution of spores to a certain extent. It would, therefore, seem that the only

plausible explanation which can be offered, in the light of our present knowledge, for the solution of this problem, is the supposition that under certain natural conditions unfavorable to the development of the anthrax bacillus an attenuation of its virulence takes place, and then, again, an increase of virulence as the condition becomes more favorable—a result which can be produced by artificial means. But whether this actually occurs we do not know.

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 prepared 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, according to Koch, Gaffky, and Löffler. 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 in France, Hungary, and Russia. Statistics collected by Chamberland of the results of twelve years’ use of this

method in France show that 3,300,000 sheep have been thus inoculated. Of these 1 per cent. only have died from anthrax, either during or after treatment; whereas the mortality previous to the introduction of this method was 10 per cent. on the average. Of 438,000 cattle inoculations only 0.33 per cent. have died; the previous mortality from anthrax was 5 per cent. These figures would seem to indicate the practical value of Pasteur's method of inoculation, notwithstanding the arguments which have been put forward in opposition to it. It is, however, not unattended with danger, as some of the animals succumb to the after-effects of the attenuated culture.

Differential Diagnosis. The differential diagnosis of the anthrax bacillus is ordinarily not difficult, as this organism presents morphological, biological, and pathogenical 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 inoculated in nutrient gelatin and agar in Petri plates and in mice.

Among other bacteria which may possibly be mis-

taken for anthrax bacilli are the bacillus subtilis and the bacillus of malignant oedema. 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 anaërobie, 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. According to Cornil and Babes, some of these cases may possibly be caused by organisms other than the bacillus of anthrax.

BACILLUS ANTHRACIS SYMPTOMATICI.

(*Bacillus of Symptomatic Anthrax.*)

Like the bacilli of anthrax and of malignant oedema, 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—affected with what is known as “black leg,” “quarter evil,” or symptomatic anthrax (German, *rauschbrand*; French, *charbon symptomatique*), a disease which prevails in certain localities in summer, and is characterized by a peculiar emphysematous swelling of the subcutaneous tissues and muscles, especially over the quarters.

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 an-

thrax bacilli in culture and the bacilli of malignant oedema in the bodies of animals are frequently seen to do. Under the hanging drop the bacilli are observed to be actively motile, and in stained preparations 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.

Biological Characters. Like the bacillus of malignant oedema this is also 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.

Growth on Agar. The colonies on agar are somewhat more compact than those of malignant oedema, 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 oedema), 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, 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 oedema, 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 the 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 experimental infection.

Symptomatic anthrax, like anthrax and malignant oedema, is a disease of the soil, but it shows a more limited endemic distribution than the first, 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 devel-

opment outside of the body like anthrax. In the form of spores, however, reproduction may take place; and by contamination with these, through deep wounds acquired by animals in infected pastures, the disease is spread. Possibly also it may originate through infection of the mouth and by feeding—which would account for the cases of symptomatic anthrax occurring in stalled cattle (Hafner).

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 intravenous inoculations; or, in guinea-pigs, by inoculations with bouillon cultures which have been kept for a few days and as a result have lost their original virulence, or with cultures kept in an incubating oven at a temperature of 42° to 43° C.; or by inoculations made into the extremity of the tail; or by inoculations with filtered cultures, or with cultures sterilized by heat. For the production of immunity in cattle, Arloing, Cornevin, and Thomas recommend the use of 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 in it. Two vaccines are prepared, as in anthrax—a stronger vaccine by exposure of a portion of the powder to a temperature of 85° to 90° C. for six hours, and a weaker vaccine by exposure for the same time to a temperature of 100° to 104° C. Inoculations made with this attenuated virus (into the end of the tail)—the weakest first and later the stronger—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 of Hafner, Luchanka, Hess, Strebel, etc. (1885-93), 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.

To recapitulate briefly, the principal points of differentiation between this bacillus and the bacillus of malignant œdema, which it closely simulates, are 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.

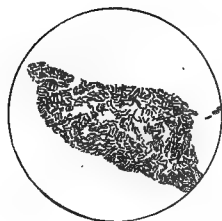
CHAPTER XXXIII.

SPIRILLUM CHOLERÆ ASIATICÆ (KOCH'S COMMA BACILLUS OF ASIATIC CHOLERA).

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 in greater number, it was said, the more acute the attack. Koch also demonstrated an invasion of the mucosa and its glands by the comma bacilli. 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. During his stay in India, in Egypt, and at Toulon, Koch had examined over one hundred cases, and other investigators confirmed his statements. Numerous control observations made upon other diarrhœic dejecta and upon normal stools were negative; the comma bacillus was found in choleraic material only, or in material contaminated by cholera. Soon other observers, however, described comma-shaped organisms of non-

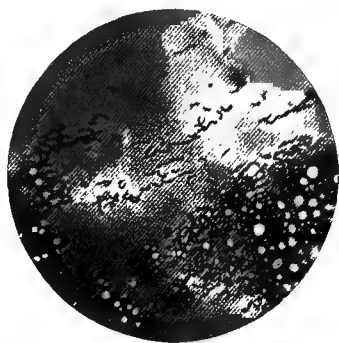
choleraic origin; Finkler and Prior, for instance, found them in the diarrhœic stools of cholera nostras, Deneke in cheese, Lewis and Miller in saliva. All of these organisms, however, differed in many respects from Koch's comma bacillus, and since then it has been proved that none of them was affected by the specific serum of animals immunized to cholera; and gradually the exclusive association of Koch's vibrio with cholera

FIG. 74.



Contact smear of colony of cholera spirilla from agar. $\times 700$ diameters. (DUNHAM.)

FIG. 75.



Contact spirilla preparation from plate culture of cholera. $\times 800$ diameters. (DUNHAM.)

became almost generally acknowledged. It is now regarded by bacteriologists everywhere to be the specific cause of Asiatic cholera.

Morphology. Curved rods with rounded ends which do not lie in the same plane, from 0.8μ to 2μ 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 junction of two vibrios S-shaped forms are produced, and under unfavorable

conditions of growth they may develop into long, spiral filaments, which may consist of numerous spiral turns in which it is impossible to recognize any connection with the individual elements of which they are made up. In stained preparations the spiral character of the long filaments is often obliterated, or nearly so. Under favorable conditions of growth—that is, when the growth is rapid—the short-curved or almost straight forms are commonly observed (Figs. 74 and 75). In old cultures involution forms are frequent.

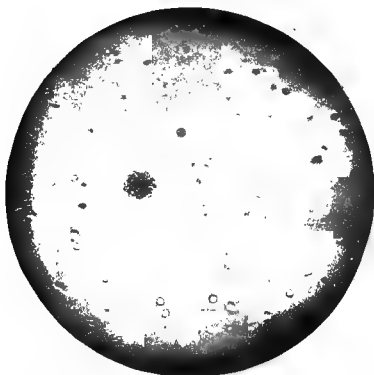
Stains with the aniline colors usually employed, but not as readily as many other bacteria; an aqueous solution of carbol-fuchsin is recommended as the most reliable staining agent with the application of a few minutes' heat. It is decolorized by Gram's method. The motile organs exhibit one or two long, fine, spiral flagellæ attached to one end of the rods.

Biological Characters. An aërobic (facultative anaërobic), liquefying, motile spirillum. Grows readily in the ordinary culture media, best at 37° C., but also at the room-temperature (22° C.); does not grow at a temperature above 42° or below 8° C. Does not form spores.

In *gelatin plate cultures*, at 22° C., 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, remains at first clear, then becomes cloudy, mostly gray, as the result of the growth of the colonies. In many cases after a time concentric rings, which increase from day to day, appear

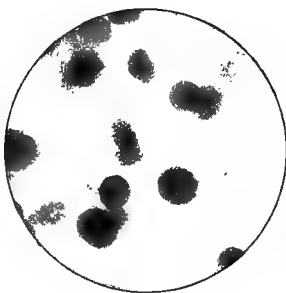
in the zone of liquefaction. (See Figs 76 and 77.) Examined under a low-power lens, at the end of sixteen to twenty-four hours, the colonies appear as small, light yellow, round, coarsely granular disks, with a more or less irregular outline. In many cases at this stage an

FIG. 76.



Cholera colonies in gelatin; twenty-four hours' growth. (DUNHAM.)

FIG. 77.

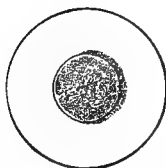


Cholera colonies in gelatin; thirty-six to forty-eight hours' growth. \times about 30 diameters.

ill-defined halo is seen to surround the granular colony, which by transmitted light has a peculiar reddish tint. The older the colonies become the more the granular structure increases, until a stage is reached when the surface looks as if it were covered with little fragments of broken glass (Koch). Liquefaction continues around the colonies, their structure appears fissured and coarsely granular in texture, and occasionally a hair-like border is formed at the periphery (Fig. 78), or a gray transparent zone, until the entire colony breaks up into fragments. 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 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;

FIG. 78.

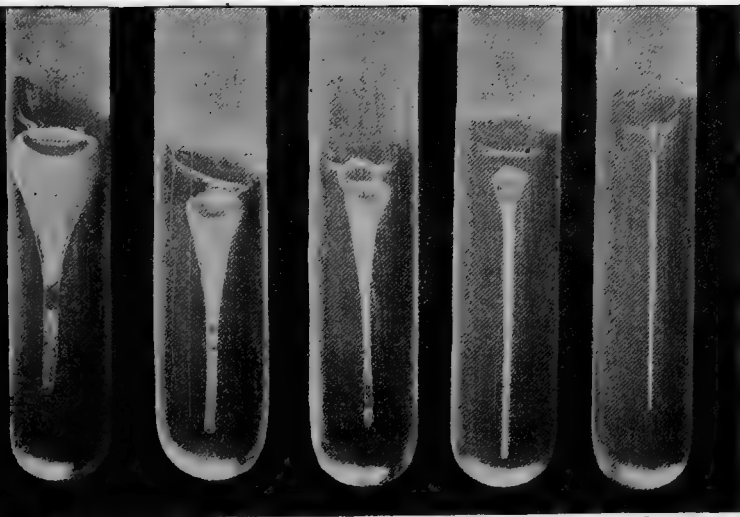
Cholera colony in gelatin. $\times 30$ diameters. (DUNHAM.)

in from eight to fourteen days the upper two-thirds of the gelatin is completely liquefied. (See Fig. 79 and Fig. 31, page 230.) Freshly isolated cholera vibrios liquefy gelatin more rapidly than old laboratory cultures; a certain variation in the characteristic liquefaction of the gelatin even in fresh cultures under some circumstances should be borne in mind in making a diagnosis. Such variations in cultural peculiarities occur also with other bacteria, and only the sum of all the characteristics taken together enables a positive diagnosis to be established.

Upon the *surface of agar* the comma bacillus develops a moist, shining, grayish-yellow layer. *Blood-serum* is rapidly liquefied at the temperature of the incu-

bator. In *bouillon* the growth is rapid and abundant; in the incubator at the end of ten to sixteen hours the liquid is diffusely clouded, and on the surface a wrinkled membranous layer is often formed. In gen-

FIG. 79.



A characteristic series of cholera cultures in gelatin; one, two, three, four, and six days' growth. (DUNHAM.)

eral 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 the presence of vegetable acids, and grows upon potatoes, in the incubator only, which have a slight acid reaction. Abundant development occurs in *bouillon* which has been diluted with eight to ten parts of water and in

simple peptone solution, and it has been shown by experiment that it also multiplies to some extent in sterilized river or well-water, and preserves its vitality in such water for several weeks or even months. Koch found in his early investigations that rapid multiplication may occur upon the surface of moist linen, and also demonstrated the presence of this spirillum in the foul water of a tank in India which was used by the natives for drinking purposes.

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.

Temperature is also of considerable importance in the growth of cultures. Active growth does not begin until a temperature of 22° to 25° C. is reached, though the optimum growth is between 30° and 40° C.

The vitality of cultures of the comma bacillus is quickly destroyed by desiccation. If a culture be spread on a cover-glass and exposed to the action of the air at room-temperature the bacilli are dead at the end of two or three hours, unless the layer of culture is very thick, when it may take twenty-four hours or more to kill all the bacilli. This fact 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 comma bacilli no longer contained the living organisms; in Berlin canal-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. Even in cultures the comma bacillus is one of the shorter-lived bacteria. They have been observed, however, in pure bouillon cultures for three to four months, and in agar cultures for six months, and occasionally in one-year-old cultures when they were protected from desicca-

tion. In these they occurred only in involution forms.

The comma bacillus is killed by exposure to moist heat at 60° C. in ten minutes. The bacilli have been found alive in ice kept for a few days, 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, according to Pfuhl's experiments, 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. The disinfecting action of mineral acids, particularly of sulphuric acid, has been advantageously employed for the disinfection of entire systems of water-works into which cholera bacilli had gained access.

Pöhl, Bujivid, and Dunham have shown that 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. Sal-kowski and Petri then demonstrated that the cholera bacilli produced in thin bouillon cultures, along with indol, nitrites by reduction from the nitrates con-

tained in small quantities in the culture media; and showed that it is the setting free of nitric acid, upon the addition of sulphuric acid to the culture, which gives with 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.

Pathogenesis. Since none of the lower animals is naturally subject to cholera, nor has ever contracted the disease during the prevalence of an epidemic or as the result of the ingestion of food contaminated with choleraic excreta, there is no reason to expect that inoculations of pure cultures of the spirillum, either subcutaneously or by the mouth, will give rise in animals to a typical attack of cholera. It has been shown, more-

over, 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. Despite the small prospects of success, however, from animal experiments, these have been undertaken again and again, until finally a method was found by which at least similar processes have been produced in test animals by inoculation of pure cultures of the cholera vibrio. 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 grammes 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 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. Koch experimented in this way on about one hundred guinea-pigs. These results, however, are somewhat

weakened by the fact that experiments made with some other bacteria—viz., those isolated by Finkler and Prior, Deneke, and Miller, and morphologically similar to the comma bacillus of Koch—occasionally produced death when introduced in the same way into the small intestines of guinea-pigs; but while only twelve out of fifty-one animals died when injected with cultures of these last-mentioned bacteria, in the cholera experiments there was 90 per cent. of deaths, and when larger doses were administered all of the animals died. Control experiments made with many other bacteria gave negative results. Intraperitoneal injections of larger quantities of pure cholera cultures also often produce death in rabbits and mice.

There are several cases on record which furnish the most satisfactory evidence that the cholera bacillus 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 cholera 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, tympanitis, and great prostration. His voice became hoarse, and the secretion of urine was somewhat dimin-

ished, this condition lasting for several days. In both cases the cholera spirillum was obtained in pure culture from the dejecta. Another instance is reported by Metschnikoff, in Paris, of a man who became infected experimentally. In this case the algid stage of cholera was produced, with complete suppression of urine, cramps in the legs, contraction of the extremities, and collapse, the man's life being saved only with difficulty. Finally, there is the case of Dr. Oergel, of Hamburg, who accidentally, while experimenting on a guinea-pig, had 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 cholera cultures of producing the disease; and yet Strickler and Hasterlick (Vienna, 1893) report negative results from experiments on the human subject. This only goes to show, however, that in cholera, like other infectious diseases, there is an individual susceptibility. It is also possible that the cultures used for experimentation may have lost in virulence, as cholera cultures are so liable to do when kept for any length of time.

Cholera Toxin. 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 finds that recent aerobic cultures of the cholera spirillum contain a specific toxic substance which is fatal to guinea-

pigs in extremely small doses. This substance stands in close relation with the bacterial cells, and is perhaps an integral part of them. The spirilla may be killed by chloroform, thymol, or by desiccation without apparent injury to the toxic power of this substance. It is destroyed, however, by absolute alcohol, by concentrated solutions of neutral salts, and by the boiling temperature. Secondary toxic products are formed which have a similar physiological action, but are from ten to twenty times less potent. Similar toxic substances were obtained by Pfeiffer from cultures of Finkler and Prior's spirillum and from the spirillum Metschnikovi.

Cholera Immunity. 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. He attributed this to the presence in the serum of convalescents from cholera of antitoxic substances which neutralized the poison produced by the cholera vibrios, in the same manner as the antitoxins of diphtheria and tetanus. Pfeiffer, on the other hand, maintained that the serum contained bactericidal substances which killed the bacilli so rapidly when injected into the animal that they did not have time to produce their specific poison, and that thus the death of the animal was prevented.

The serum is now known to be feebly antitoxic and strongly bactericidal. 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 exist also 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 alone to cholera. The discovery, moreover, of this specific reaction of the blood-serum of immunized men 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.

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, 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 then either not produced or 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 toxin is not produced to any extent by the spirilla. In no stage of the disease are living cholera spirilla found in the organs of the body or in the secretions.

From this fact and other known properties of the cholera bacillus, 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), and only these dejections, therefore, and objects contaminated by them, such as bed and body wash, floors, vaults, soil, well-water and river-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; 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 have become contaminated by the dejections of cholera patients.

But, like 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 found, for example, in 14 out of 17 persons belonging to the families of 7 cholera patients, 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.

Within the last few years Haffkine, in India, has succeeded in producing an artificial immunity against cholera infection by means of subcutaneous injections of cholera cultures. In over 200,000 persons whom he has inoculated the results obtained would undoubtedly seem to show a distinct protective influence in the preventive inoculations. And Kolle has found that the blood-serum of persons inoculated by Haffkine's method gave a similar reaction to that of persons who had recovered from cholera.

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 overeating, etc., may act as contributing causes to the disease. Other predisposing causes are general debility from poverty, hunger, disease, etc.

Varieties and Variations of the Cholera Bacillus. Cunningham, as a result of researches made in Calcutta (1891), arrives at the conclusion that Koch's comma bacillus cannot be accepted as the specific etiological agent in this disease: First, as in many undoubted cases of cholera he has failed to find comma bacilli; second, because in one case he found three different species; and, third, because in one case the indol reaction could not be obtained. Since Cunningham's investigations

many other observers have reported finding varieties of the comma bacillus. Only a few of these can be here mentioned, of which there is any certainty that they were derived from true cholera cases. Thus Friedreich has accurately described and photographed a series of forms which, however, vary but little from the original type. But more interesting than the reports of varieties are the observations of the variability of the cholera bacillus. Claussen, in Esmarch's institute, isolated from fresh cholera stools vibrios which presented in plate cultures a different appearance of the colonies, which showed a tendency to disintegrate and having an irregular border. The nitrosoindol reaction was absent; bouillon cultures were non-pathogenic to guinea-pigs, and stick cultures grew slowly and uncharacteristically. On repeated inoculation, however, a guinea-pig died after the injection of 1 c.c. of a bouillon culture; in the peritoneal exudate and even in the blood characteristic bacilli were found and the cultures gave the indol reaction. Celli and Santori, in Rome (1893), isolated from the stools of many typical cholera cases a vibrio which they called *vibrio romanus*, which was non-pathogenic for animals, gave no indol reaction, did not coagulate milk, and at 37° C. grew neither in bouillon nor on agar. After cultivation for nine months it gave the indol reaction and grew at 37° C., but was still almost non-pathogenic. Bordoni-Uffreduzzi and Abb cultivated from a typical cholera case a short vibrio which liquefied gelatin very rapidly and presented an abnormal growth, and gave a yellow growth on potato, but which on continued cultivation became more and more like the cholera spirillum. Variations even greater than occur in these varieties of cholera spirilla are met with among diphtheria bacilli.

Plan of Procedure for the Biological Diagnosis of the Cholera Vibrio. 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 the 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.

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, 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.

¹ These direct microscopical 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.

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).

14. Make gelatin tube cultures daily for five or six days, to study shape of growth along the line of puncture to preserve the culture.

B. Suspected water.

Add to 500 c.c. or 1 litre of the water to be examined in a flask half-full 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*.

PFEIFFER'S SERUM REACTION. 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, or by watching the action of the spirillum in the hanging drop when added to a dilution of the above mentioned serum, so as to note whether immobilization and clumping occurred.

CHAPTER XXXIV.

SPIRILLA RESEMBLING THAT OF CHOLERA—THE SPIRILLUM OF RELAPSING FEVER.

SPIRILLUM OF FINKLER AND PRIOR.

FINKLER and Prior, in 1884, obtained from the feces 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.

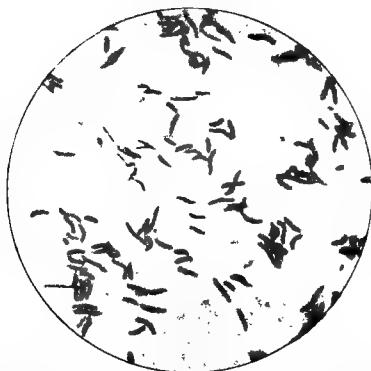
Morphology. More or less curved rods with an average length of 2.4μ and a breadth of 0.4 to 0.6μ , somewhat longer and thicker than the spirillum of Asiatic cholera and not so uniform in diameter, the central portion being usually wider than the pointed ends; forms sometimes S-shaped and spiral filaments, which are not as numerous, and are usually shorter than those formed by the cholera spirillum. Examined in the hanging drop they are seen to be actively motile. A single flagellum is attached to one end of the curved segments. In unfavorable media involution forms are common.

Stains with the usual aniline colors.

Biological Characters. 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, which

under the microscope are seen to be finely granular and yellowish or yellowish-brown in color; the colonies are round with more sharply defined border, less coarsely granular and darker in color than those of 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 numerous. The surface colonies sink quickly into the gelatin

FIG. 80.

Spirillum of Finkler and Prior. $\times 1100$ diameters.

and present a darker peripheral zone. The differentiation between the Finkler and Prior and cholera spirilla can readily be made in the earlier stages of their growth. Later on, and especially when the cholera colonies are the older, the diagnosis is not so easy. 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 is already seen after forty-eight hours, which is filled with a cloudy liquid. There is no bubble formation. The liquefac-

tion 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 with the cholera vibrio; a slimy, whitish-yellow layer covering the entire surface is quickly developed. Upon *potato* this spirillum grows at the 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. Cultures of the Finkler and Prior spirillum give off a strong putrefactive odor; in media containing sugar, according to Buchner, an acid reaction is produced as a result of their growth; they do not form indol in peptone solutions; and they have a greater resistance to desiccation than the cholera spirillum. The absence of agglutination with a dilution of the serum of an animal immunized to the cholera spirillum is a valuable differential sign.

Pathogenesis. When injected into the stomach of guinea-pigs, after previous injection of a soda solution and opium, the Finkler and Prior spirillum is somewhat pathogenic for these animals; but a smaller proportion die from such injections than from those of the fresh cultures of cholera. At the autopsy the intestine is pale, and its watery contents, which contain the spirilla in great numbers, have a putrefactive odor.

This organism has been found in the dejections of some healthy persons and of persons affected with diarrhœa or cholera nostras. It does not seem to have any etiological relation, however, with this disease in man,

as since its discovery, though repeatedly sought for, it has seldom been found by subsequent investigators.

In 1884, Miller observed a curved bacillus in a hollow tooth, which from its behavior in microscopical preparations, in cultures and animal experiments, is probably identical with the Finkler and Prior spirillum; and other very similar spirilla have been found by others.

DENEKE'S CHEESE SPIRILLUM.

Obtained by Deneke (1885) from old cheese, but since then rarely met with. Morphologically and culturally it shows greater similarity to Koch's comma bacillus than the Finkler and Prior spirillum, but can be readily differentiated from it also.

Morphology. Curved rods and long spiral filaments, somewhat more slender than the cholera spirillum, the turns in the spiral threads being lower and closer together. Has a single flagellum attached to one end.

Stains with the usual aniline colors.

Biological Characters. An aërobic and facultative anaërobic, liquefying, motile spirillum. Does not form spores. Upon *gelatin plates* small, punctiform colonies are formed at the end of twenty-four hours, which when slightly magnified are seen to be circular in shape, with sharply defined border and of a greenish-brown color in the centre and paler toward the margins. Later, when liquefaction has commenced, the sharp contour is often lost. The liquefaction progresses more rapidly than with the cholera bacillus, but not so energetically as with the spirillum of Finkler and Prior. In *gelatin stick cultures* after forty-eight hours a stocking-like pouch is developed, the spirilla sinking to the bottom

of the liquefied gelatin in the form of a coiled mass, while a thin, yellowish layer forms upon the surface; complete liquefaction usually occurs in about two weeks. Upon the surface of *agar* a thin, yellowish layer is developed. *Blood-serum* is rapidly liquefied. The indol reaction in peptone solutions is absent.

Pathogenesis. Somewhat pathogenic for guinea-pigs when inoculated by Koch's method with previous administration of soda solution and laudanum.

It is probable that this organism, from the locality in which it is found and its behavior, is a saprophyte.

SPIRILLUM METSCHNIKOWI.

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; it forms curved rods with rounded ends and spiral filaments, the curved segments being somewhat thicker, shorter, and often more decidedly curved than the comma bacillus. 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. In old cultures beautiful long spiral filaments may be seen.

Stains with the usual aniline colors, but not by Gram's method.

Biological Characters. An aërobic, liquefying, motile spirillum. Upon *gelatin plates* the vibrio Metschnikovi 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. Under the microscope the colonies appear as yellowish-brown granular masses, which are in active movement, and the margins are surrounded by a border of radiating filaments. 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 pure sulphuric acid to twenty-four-hour peptone cultures a distinct nitroso-indol reaction is produced. *Milk* is coagulated and acquires a strongly acid reaction. The spirillum does not clump and lose its motility with the diluted serum from an animal immunized to cholera.

Pathogenesis. The vibrio Metschnikovi is pathogenic for fowls, pigeons, and guinea-pigs. A small quantity of a culture injected into the breast muscles of 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; the spleen is not enlarged,

rather diminished in size, and the organs generally are normal in appearance. In the watery fluid large numbers of spirilla are found; they are found in the blood of pigeons always, but only in the blood of young fowls. A few drops of a pure culture inoculated subcutaneously in pigeons cause their death in eight to twelve hours. According to Gamaleïa, fowls may be infected by giving them food contaminated with the cultures of the spirillum, but pigeons resist infection in this way.

Infection may also be produced by way of the mouth by Koch's method, a solution of carbonate of soda and laudanum having been previously administered. The animals then die with symptoms of acute gastro-enteritis; the intestines are found to be highly inflamed and their liquid contents contain numerous spirilla. 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 cholerae. Gamaleïa has claimed that by passing the cholera spirillum of Koch through a series of pigeons, by successive inoculation, its pathogenic power is greatly increased, and that when sterilized cultures of this virulent variety of the comma bacillus are injected into pigeons they become immune against the pathogenic action of the vibrio Metschnikovi, and the reverse. But Pfeiffer has shown that this statement is not founded upon fact. The pathogenic action of the vibrio Metschnikovi 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 vibrios, the so-called "water vibrios," have been found while looking for the cholera bacillus, the identity or variation of which from the spirillum of cholera it has been extremely difficult to determine, as morphological, biological, and pathogenical examinations have led to no positive results.

SPIRILLUM OBERMEIERI (Spirillum of Relapsing Fever).

First observed by Obermeier (1873) in the blood of persons suffering from relapsing fever.

Morphology. 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.

Stains readily with the ordinary aniline colors, especially with fuchsin, Löffler's solution of methylene-blue and Bismarck-brown. Does not stain by Gram's method.

Biological Characters. A motile spirillum which has not been cultivated in artificial media. Spore formation has not been demonstrated. In fresh preparations from the blood the spirillum exhibits active progressive movements accompanied by very rapid rotation in the long axis of the spiral filaments or by undulating movements. The spirilla are found exclusively in the blood and spleen of persons suffering from relapsing fever, never in the secretions, and only during the fever, not in the intermissions, or at most singly at the beginning of an attack. When preserved in blood-serum or a 0.5 per cent. solution of salt they continue to exhibit active movements for a considerable time.

Efforts to cultivate this spirillum in artificial culture media have thus far been unsuccessful, although Koch has observed an increase in the length of the spirilla and the formation of a tangled mass of filaments.

Pathogenesis. Inoculation experiments have been successfully made on man and monkeys. Monkeys when inoculated with human blood containing the spirilla take sick after about three and a half days, but show only the initial febrile attack; no relapses, such as are characteristic of the disease in man. The organisms are found in the blood, and at the height of the fever in the other organs on autopsy. Extirpation of the spleen renders the disease more dangerous for monkeys.

Blood from one animal, taken during the attack, induces a similar febrile paroxysm when inoculated in another monkey. One attack does not preserve the animal experimented on from a second attack (Koch and Carter).

Very little is known bacteriologically of this disease, but from the fact that these peculiarly shaped organisms are constantly and exclusively found in relapsing fever, and that the disease can be transmitted to monkeys by inoculating them with the blood containing the spirilla, it may be assumed that they are the true cause of the disease.

CHAPTER XXXV.

GLANDERS BACILLUS.

BACILLUS MALLEI (Bacillus of Glanders).

THIS bacillus was discovered and proved to be the cause of glanders by isolation in pure culture and communication to animals by inoculation, by several bacteriologists almost at the same time (1882), viz., by the investigations of Löffler, Schütz, Israel, Bouchard, Charrin, Weichselbaum, Kauzfeld, and Kitt. It is found 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 0.25μ to 0.4μ broad and from 1.5μ to 3μ long; usually single, but sometimes united in pairs, or growing out to long filaments, especially in potato cultures. Frequently breaks up into short, almost coccus-like elements (Fig. 81).

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. Löffler 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.

FIG. 81.

Glanders bacilli. Agar culture. $\times 1000$ diameters.

Biological Characters. 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. It 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, was effectual in destroying its vitality. As a rule, the bacilli do not grow after having been preserved in a desiccated condition for a week or two; in distilled water they are also quickly destroyed. The bacillus does not grow in infusions of

hay, straw, or horse-manure, and it is doubtful whether it finds conditions in nature favorable to a saprophytic existence. 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, and the potato about it acquires a greenish-yellow tint. In *bouillon* it causes diffuse clouding, with ultimately the formation of a more or less ropy tenacious sediment. *Milk* is coagulated with the production of acid. It grows on media possessing an acid reaction, and both with and without oxygen.

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 the bacillus mallei are injected into horses and 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 mucus; 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; but the discharge from the nostrils or from an open ulcer contains comparatively few bacilli, and these being associated with other bacteria which grow more readily on the culture media than the *bacillus mallei*, it is not easy to obtain pure cultures by the plate method from such material, and here animals 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 caseous contents soon develops; then ulceration of the skin takes place, and a chronic purulent ulcer is formed. 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 mem-

brane, which characterize the disease in the horse, rarely result from inoculation of the guinea-pig. The process is often prolonged, or it remains localized on the skin. Guinea-pigs succumb more rapidly to intraperitoneal injection, usually in from eight to ten days, and in males the testicles are invariably affected.

Glanders occurs as a natural infection only in horses and asses; the disease is occasionally communicated to man by contact with affected animals, and 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 the horse, the disease may be localized in the nose (glanders) or beneath the skin (farcy). 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. These nodular masses tend to break down rapidly, and on the mucous membrane form ulcers, while beneath the skin they form abscesses. The glanders nodules may also occur in the internal organs. An acute and chronic form of glanders may be recognized in man, and an acute and a chronic form of farcy. The disease is fatal in a large proportion of cases. It is transmissible also from man to man. Washer-women 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 sometimes in blood; it may occasionally be found in the secretions of healthy glands, as in the urine, milk, and saliva, and also in the foetus 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 (Semmer and Babes). Apparently healthy horses, 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 into susceptible animals.

Several attempts have been made by investigators to produce artificial immunity against glanders, but so far with unsatisfactory results. According to Strauss, dogs may be protected by intravenous inoculations of small quantities of living bacilli against an injection with large 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*. 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. It is made in the same way as Koch's crude tuberculin from the tubercle bacillus cultures.

Differential 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. As a rule, it is necessary to make animal tests of these discharges by inoculating susceptible animals, as guinea-pigs and mice, and then from these 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 be made within two or three days from the tumefaction of the testicles, which become red and 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. This is particularly frequent when field mice are used for the tests; but if pure matter can be obtained, as from the lymphatic glands of the horse, this method is entirely satisfactory.

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, which should be 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., or even 4° C., 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 oedema 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 cedematous tumor, and the oedema, 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.

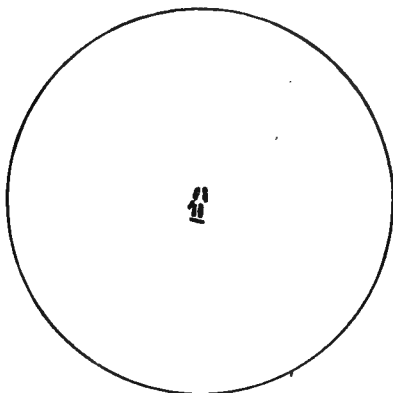
CHAPTER XXXVI.

BUBONIC PLAGUE BACILLUS—YELLOW FEVER
BACILLUS—WHOOPING-COUGH BACILLUS.

BACILLUS OF BUBONIC PLAGUE (*Bacillus Pestis*
Bubonicæ—Kitasato ; *Bacterium Pestis*).

DISCOVERED simultaneously by Kitasato and Yersin (1894) during an epidemic of the bubonic plague in China. It is found in large numbers in the pus from

FIG. 82.



Bacillus of bubonic plague. $\times 1000$ diameters.

the buboes characteristic of this disease and in the lymphatic glands; more rarely in the internal organs and in the blood, in which it occurs in acute hemor-

rhagic cases and shortly before death. It also occurs in the feces of men and animals.

Morphology. Short thick rods, with rounded ends, frequently occurring in short chains and often surrounded by a capsule. When obtained from cultures the bacilli present considerable spherical enlargement (Fig. 82).

Stains readily with the ordinary aniline dyes, the ends being usually more deeply colored than the central portion; does not stain by Gram's method.

Biological Characters. An aërobic, non-motile bacillus. Does not form spores. Grows on the usual culture media. Does not liquefy gelatin. Grows best on *blood-serum* in the incubator, the growth appearing on the surface after twenty-four to forty-eight hours, in the form of white, moist, transparent and iridescent colonies. It grows rapidly on *glycerin-agar*, forming a grayish-white surface growth. In *bouillon* a very characteristic appearance is produced, the culture medium remaining clear while a granular or grumous deposit forms on the walls and on the bottom of the tube.

Pathogenesis. This bacillus is pathogenic for rats, mice, guinea-pigs, monkeys, rabbits, 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 oedema, 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, and thus its extension is

facilitated. During epidemics, rats, mice, and flies, in large numbers, become infected and die, and the disease is apparently transmitted through them to man. The organism is found in the feces of sick animals, in the dust of infected houses, and in the soil.

The virulence of the bacilli in cultures and in nature seems to vary considerably, and is rather rapidly lost 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.

Yersin, Calmette, and Borrel have succeeded in immunizing animals against the bacillus of bubonic plague by inoculation, 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 by Yersin to the treatment of bubonic plague in man, with very 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 usually 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.

Haffkine, in India, has recently 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, 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 great mortality at times, may finally be greatly restricted or even stamped out altogether.

BACILLUS ICTEROIDES (Bacillus of Yellow Fever).

In 1897 Sanarelli announced the discovery of a micro-organism which he claimed to be the specific cause of yellow fever. This he called the "bacillus icteroides." It is found in the circulating blood and in the tissues of yellow fever patients.

Morphology. Short rods with rounded extremities, single or united in pairs in cultures and in groups in the tissues, from 1μ to 2μ in length, and generally two to three times longer than broad. Somewhat polymorphous. It resembles the colon bacilli.

Stains readily with the ordinary aniline dyes, but not by Gram's method.

Biological Characters. A motile, facultative anaërobic, non-liquefying bacillus. Does not form spores as far as known. Grows readily in all the ordinary culture media, at the room-temperature, but best at 37° C. in the incubator. On *gelatin plates* it forms rounded colonies, transparent and granular. It never liquefies gelatin. In *bouillon* the bacillus grows quickly, without forming either a pellicle or deposit. On *blood-serum* its growth is almost imperceptible. Cultures on *agar* are characteristic, according to Sanarelli. When the colonies grow in the incubator they present an appearance that does not differ from many other species; they are rounded, of a slightly iridescent gray color, transparent, even in surface, and regular in outline. Grown at the room-temperature from 20° to 22° C., they appear like drops of milk, opaque, projecting, and with pearly reflections, completely distinct from those grown in the incubator. These different modes of evolution Sanarelli considers to be an important diagnostic point; first exposing the cultures for from twelve to sixteen hours to the temperature of the incubator, and afterward for twelve to sixteen hours more to the temperature of the air.

The bacillus *icteroides* ferments glucose and saccharose, but does not coagulate milk; produces little indol, and is quite resistant to desiccation; it dies in water at 60° C., or after being exposed for seven hours to the sunlight, and lives for a long time in sea-water.

Pathogenesis. It is pathogenic for the greater number of the domestic animals; but birds are completely refractory. According to the discoverer the dog lends

itself particularly to experimentation with this organism. The virus should be injected into a vein. The lesions found after death are said to be almost identical with those in human yellow fever cadavers. There is fatty degeneration of the liver and kidneys, accompanied by acute parenchymatous nephritis. The digestive apparatus shows lesions of hemorrhagic gastro-enteritis. The bacilli are found in the blood and the organs in variable quantity and in a state of absolute purity; at times, they may be associated with the *B. coli* and the streptococcus.

The disease may be transmitted experimentally even by the respiratory tract to rabbits and guinea-pigs; the bacteriological examination of these cases shows, at least, the existence of toxic processes similar with that which takes place in man. The toxin is obtained by filtering twenty to twenty-five days' old cultures in broth. It withstands a temperature of 70° C., but is sensibly weakened by boiling.

According to Sanarelli, infection in the human subject does not take place by the digestive but by the respiratory tract; and he suggests that the common moulds of the atmosphere may constitute protectors of the bacillus icteroides.

Sanarelli has also prepared a protective or curative serum for the treatment of the disease, which he calls "anti-amarylic serum." This serum has not been sufficiently tested as yet to form any definite conclusions as to its value; but because of its not being an antitoxin, it does not tend to overcome the toxins of yellow fever produced in the system, and depends for its curative and prophylactic properties upon its germicidal influence. Hence, it is argued by Sanarelli that

its use will be absolutely negative in cases in which an amount of toxin has been produced sufficient to destroy life. He, therefore, insists upon the early use of the serum, and thus the destruction of the organism before it has elaborated the fatal proportion of its toxin. It is claimed that in thirty-one cases treated with the serum the mortality was only 32 per cent., whereas in South America, where the treatment was applied, the mortality of yellow fever often rises to 50 per cent. (Sanarelli).

Since Sanarelli's supposed discovery a number of investigations have been made into the causal relation of this organism to the disease, some of which seem to cast considerable doubt upon its being the specific cause of yellow fever, while others are in its favor. Novy, in a recent paper (September, 1898), comes to the conclusion, after an exhaustive examination into this subject, that the Sanarelli bacillus belongs to the typhoid group, and that it is not the cause of yellow fever, which is yet to be worked out.

Novy's chief objection to this bacillus rests upon the fact that yellow fever is stopped by a frost and that this bacillus is not injured by much greater cold. It is perfectly possible, however, that the infection is carried in some indirect way, as by insects, and that the carriers of infection are affected by the cold, and so the dissemination of the poison is prevented. The long immunity conferred by an attack and the peculiar effect of cold on the spread of the disease are nevertheless difficult to explain by means of the known characteristics of this bacillus. In September a report by Geddings and Wasdin appeared which favored the claims of Sanarelli, they finding the bacilli in almost every case of yellow fever, and not in any which were

not yellow fever. According to them the lungs were the earliest and the chief seat of the lesions. Their report adds to the mystery of the effect of cold on stopping the spread of the disease, for nearly all respiratory diseases due to bacteria tend to increase in cold weather, and certainly their spread is not stopped immediately. This bacillus can as yet be considered as only the possible cause of yellow fever.

THE BACILLUS OF WHOOPING-COUGH.

From time to time observers have found in the sputum of persons suffering from whooping-cough small bacilli, often in great numbers. These have been studied lately especially by Koplik¹ and Czaplewski² and Hensel, who believe that these bacilli are the cause of the disease. They are small bacilli of about the size of the influenza bacillus, and grow on blood-serum and nutrient agar in tiny colonies. Mice and rabbits die after intravenous inoculations. No symptoms similar to those in man are noted. The observers differ as to the description of the bacilli, and those interested are referred to the original articles. The examination for these bacilli, if they prove to be the true cause of the disease, may prove of diagnostic importance, and also be of use in detecting sources of contagion.

¹ Centralblatt für Bact. Abth., 1 Bd. 22, p. 222

² Ibid., p. 641.

APPENDIX.

BRIEF DESCRIPTIONS OF A FEW REPRESENTATIVE PATHOGENIC MICRO-ORGANISMS WHICH ARE NOT BACTERIA.

CHAPTER XXXVII.

THE STREPTOTHRIX GROUP—FAVUS AND RINGWORM FUNGI.

THE varieties of the streptothrix group have as yet not been clearly described. Some at least are pathogenic. This group of micro-organisms while having many affinities with the bacteria, yet differs from them in many important respects which link them with the fungi. 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-hypæ, and these break up into chains of round spore-like bodies, which do not, however, have the same staining reactions nor resisting powers as true spores. The tubercle grass and diphtheria bacilli are by some believed to properly belong in the streptothrix group on account of the true branching forms developed by them under certain conditions. The best known of the streptothrix group is the *actinomyces fungus*.

STREPTOTHRIX ACTINOMYCES (*Actinomyces Fungus* ;
Ray Fungus).

This micro-organism was first described by Bollinger (1877) in the ox, in which it forms the affection known as "big-jaw." In man the disease was first described by J. Israel (1885), and subsequently Ponfick insisted upon the identity of the disease in man and cattle. To Boström we owe the most elaborate and accurate account of the structure and development of this organism.

Morphology. In both man and animals it can be seen in the pus from the affected regions as small yellowish granules from 0.5 to 2 mm. in diameter. Microscopically these bodies are seen to be made up of threads which radiate from a centre and present bulbous, club-like terminations. These club-like terminations are characteristic of the actinomyces. They are generally arranged in pairs, closely crowded together, and are very glistening in appearance. The threads which compose the central mass of the granules are from 0.3μ to 0.5μ in diameter; the clubs are from 6μ to 8μ in diameter.

The organism is *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.

Biological Characters. It grows in all the ordinary artificial culture media, but often several cultures have to be made before getting a satisfactory one. It develops at the room-temperature, and grows both with and without oxygen, but best with access of air and at the temperature of the body.

Growth on Blood-serum and Agar. Isolated colonies at first develop on the surface of these media, but on

keeping the cultures for a week or more the colonies run together and form a thick, wrinkled mass which sinks into the media. The individual colonies are yellowish to red in color, and are covered by a whitish, fluffy down, consisting of cobweb-like threads. On touching the colonies they will be found to cling close to the medium, and on forcible removal they go to pieces. On making a smear preparation the thread-like structure will be seen. In stick cultures the growth usually presents a tree-like appearance, but it varies very considerably; there may be no reddish pigmentation, and the cobweb-like threads are not always developed on the surface. Occasionally the culture on agar is colored brown.

The Growth in Bouillon. When the medium is allowed to stand perfectly still a distinct granular growth occurs, but on agitation these grains are broken up, though the liquid is never clouded. At times large flakes or a membranous film form on the surface of the medium, upon which develops the fluffy down previously described.

The Growth on Potato, On this medium the growth is somewhat slower, resulting in a thick, viscid, membranous deposit on the surface of the potato on which the same cobweb-like threads are developed. The color is yellowish-red.

The cultures are quite resistant to outside influences; dried they may be kept for a year or more; they are killed by a temperature of 75° C., the time of exposure being five minutes.

Occurrence in Animals. Actinomycosis is quite prevalent among cattle, in which it occurs endemically; it is more rare among swine and horses, and is sometimes found in man. The disease is probably not con-

tagious, but infection may result from the ingestion of vegetable products which contain the fungus. 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 micro-organism 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 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 leucocytes. Not infrequently a mixed infection with the pyogenic cocci occurs in actinomycosis.

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 experimental production of actinomycosis in animals has been followed by negative or very unsatisfactory results. 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 por-

tion of the fungus are found to have disappeared, leaving only the remains of the club-like ends, thus showing that no growth has taken place. Ponfick, Johne, Rotter, Lüning, and Hanan claim to have obtained positive results in animals, but according to Bostrom these results are not conclusive. The animals used for experimentation have been calves, swine, dogs, rabbits, and guinea-pigs, the places of inoculation being the anterior chamber of the eye, the subcutaneous intercellular tissue, the peritoneum, and the blood; and the material employed for inoculation, pus from the infected regions in animals and man; very rarely cultures.

A number of other streptothrices have been described in connection with pathogenic processes, but most of them are not well defined. They have been found in brain abscess, cerebro-spinal meningitis, pneumonic areas, and in other pathological conditions. Eppinger injected cultures into guinea-pigs and rabbits, and observed that it caused a typical pseudotuberculosis. Consolidation of portions of both lungs, thickening of the peritoneum, and scattered nodules resembling tubercles, were noted in a case of human infection as due to a streptothrix by Flexner, in which the pathological picture of the disease resembled so nearly tuberculosis in human beings that the two diseases could be separated only by the causative micro-organism in each case.

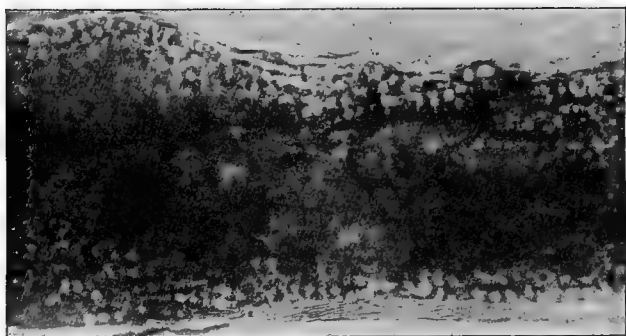
THE FUNGI.

Most of the fungi are not pathogenic and interest us merely as organisms which are apt to infect our bacteriological media. Some are, however, true parasites, and already we know that ringworm, favus, thrush, and pityriasis versicolor are caused by fungi. Only those causing ringworm and favus can be touched on here.

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 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.

FIG. 83.



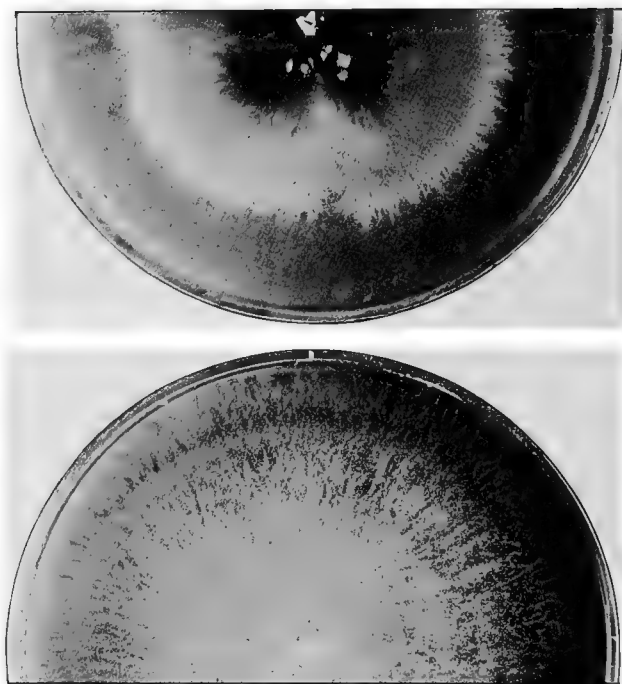
Hair riddled with ringworm fungus. *Megalosporon* variety.

According to Sabouraud, whose conclusions are based on an extensive series of microscopical 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. 83) is essentially the fungus of ringworm of the beard and of the smooth parts 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 spores appearing irregularly piled up like *zoöglæa* 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 easily separating 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 or 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. 84). Many animals are subject to the growth upon their skins of particular species of *T. megalosporon*.

FIG. 84.



These two half-plates show three-months' growth on peptone maltose agar of two megalosporon varieties of the ringworm fungus. Natural size.

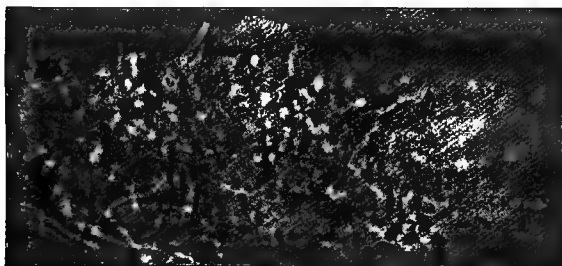
ACHORION SCHÖNLEINII (*Favus*).

Favus is due to a fungus discovered by Schönlein in 1839, and called by Remak *Achorion schönleinii*. The disease is communicated by contagion, the fungus being often derived from animals, especially cats, mice, rab-

bits, fowls; and dogs are also 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

FIG. 85.



A portion of a favus infected hair. Magnified.

hair-follicles, where they grow round the hair-seat (Fig. 85). 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 gastro intestinal irritation, which was found after death to be due to the presence

of the favus fungi in the stomach and intestine. 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.

There is some difference of opinion as to whether there is only one or several varieties of favus fungus. It was suggested by Quincke that there are three different species of favus fungus. Later investigations

FIG. 86.



Five-months' old colony of favus on peptone maltose agar. Actual size.

have apparently shown, however, that the achorion *Schœnleinii* is the only fungus of favus.

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. 86). The growth develops slowly and shows a preference to grow 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.

YEASTS (*Saccharomyces*).

These micro-organisms are of the greatest importance in brewing and baking, but as yet no important pathological lesions in man have been attributed to them, although certain recent experiments have shown that some varieties when injected are capable of producing tumors and many are pathogenic for mice. They are not uncommonly present in the air and in cultures made from the throat. They consist of round or oval cells, usually many times larger than the bacteria. They usually reproduce themselves by budding, a portion of the protoplasm budding, and finally being cut off to form a new individual.

CHAPTER XXXVIII.

PLASMODIUM MALARIÆ (MALARIAL PARASITES;
LAVERANIA)—AMCEBA COLI (AMCEBA DYSEN-
TERIÆ OF COUNCILMAN AND LAFLEUR; DYSEN-
TERIC AMCEBA).

MANY attempts have been made from time to time to discover a specific organism in malaria. As early as 1846, according to Marchiafava and Bignami, an Italian observer (Risori) suggested the possible parasitic nature of the disease. In 1880, Laveran announced the discovery of certain parasitic bodies in the blood of patients with malarial fever. He recognized that they were parasites, and raised the question whether they were amœbæ. Subsequently, influenced no doubt by the presence of the motile filaments, he suggested the term *oscillaria malarix*. Marchiafava and Celli described with great accuracy the intracorpuscular amœboid form, to which they gave the name *plasmodium*. The most important additional fact was added by Golgi, who pointed out the association of the paroxysm with the segmentation of a group of the malarial organisms. Laveran's work and the differentiation by the Italian observers of varieties of the parasite in different clinical forms of the disease have since received full confirmation, and the testimony is now unanimous in France, England, India, America, Italy, and Germany that these bodies are always present in the malarial fevers.

There is still much uncertainty with regard to the classification of the parasites. Many authors place them among the *sporozoa* in the order of the *hæmosporidia* of Danilewsky; others place them in the *sarcodinia*, and speak of them as *hæmamœbæ*. Until the matter is settled, however, they may be considered to belong to the general order of *protozoa* and to that group of organisms known as *hæmatozoa*. Parasites of the red blood-corpuscles have been met with abundantly in the blood of fish, turtles, and many species of birds.

The relation of the different forms of the malarial parasite to each other and to the varieties of the disease are still under discussion. Galgi, Marchiafava and other Italian observers hold that they are distinct varieties, not interchangeable, though closely allied biologically. Laveran, on the other hand, contends for the unity of the forms, which he regards as modifications of one polymorphic parasite. But with the present imperfect knowledge of the full life-history of the parasite the question cannot be considered as settled.

The following varieties are associated with the different forms of malarial fever :

I. **Parasite of the Simple Intermittent Fever.** (*a*) **tertian parasite** (see Plate II.). If the blood of a patient be examined during or shortly after the chill in tertian fever, inside the red blood-corpuscles, or less often free in the plasma, will be seen small, pale, hyaline amœbæ which undergo rapid changes in shape, often assuming the form of a star or of a cross. There may be no pigment visible, and to these hyaline bodies Marchiafava and Celli gave the name *plasmodia*. In a few examples scattered pigment granules may be seen in the amœbæ, usually placed near the periphery. In

dry specimens these bodies stain deeply with methylene-blue, and they are solid or vesicular in form. If the examination be made within twelve to eighteen hours after the chill the hyaline bodies are seen to have grown to occupy one-fourth to one-third of the bodies of the red cells. They are more pigmented, and the corpuscles containing them have become gradually paler and somewhat expanded. The pigment granules, which at first are small, increase in size, and the organisms show very active amœboid movements. At the end of forty-eight hours they occupy entire corpuscles, are very sluggish in their movements, and look like thin, translucent shells, and are usually devoid of color. Many of the organisms then undergo the remarkable change known as segmentation, which precedes and is associated with chills and fever. The amœboid movement ceases as well as that of the pigment granules. The latter gradually collect toward the centres of the amœbæ until they are in the form of closely packed, more or less central clumps. The protoplasm becomes more finely granular, and indistinct lines of striation are seen, which begin at the periphery. At this stage the organisms may present the appearance of rosettes. The segmentation progresses until the entire protoplasm is divided into twelve to eighteen or twenty spheres. The shell of the corpuscles containing a parasite usually bursts, and the small, rounded, hyaline bodies are set free. Each one of these little bodies consists of a translucent protoplasm, with a central, more highly refractile spot. In stained preparations, during the segmenting process, the reticulum becomes denser and sharper, and then breaks up into fifteen to twenty small spheroidal spores.

- The segmentation is regarded as a reproductive process, and these small spherical bodies are believed to be the spores which penetrate a new set of corpuscles, and so begin a new cycle of development. The pigment is discharged into the plasma, and partly taken up by the leucocytes. It is finally lodged chiefly in the spleen, liver, and lymphatic organs. The presence of the segmenting forms is invariably associated with the paroxysm. On finding them in the blood it can be predicted with certainty that a paroxysm is imminent. In quotidian fever we have to deal with two groups of tertian (or three groups of quartan) parasites, maturing on successive days; and the full-grown segmenting forms of to-day's paroxysms and the half-grown organisms of to-morrow's attack are to be found in the blood.

(b) QUARTAN PARASITE (see Plate II.). The early forms within the red blood-corpuscles are amœboid bodies, similar to those of tertian fever. Soon, however, it is noticed that the pigment is different; the granules are larger and blacker, and the amœboid movements are not so active. In their growth the parasites do not decolorize the corpuscles, which sometimes have a greenish, brassy look. From the sixty-fourth to the seventy-second hour the amœbæ have reached their full development, occupying the greater portion of the affected corpuscles; but a thin rim of colored stroma can usually be seen. Some of the corpuscles are completely filled by the parasites. The cells, as a rule, appear shrunk rather than swollen. Even at this stage a skilled observer can usually recognize the quartan from the tertian organism. The pigment granules then collect toward the centre, and

in so doing usually form distinct rays. Then, as in the tertian form, the organism begins to segment; a marginal indentation is first seen, with lines of radiation, and a beautiful rosette is formed, which segments into from six to ten, occasionally twelve, small, spherical or ovoid bodies. The character of the pigment, the smaller size of the organism, and the development are differences which separate the quartan from the tertian variety.

In the quartan malarial fever the blood may show two or more groups of parasites. There may be two groups which reach maturity on successive days, with one day interval—double quartan fever; or there may be three groups of organisms maturing on successive days, causing daily paroxysms—triple quartan fever.

II. The *Æstivo-autumnal Parasite* (see Plate II.). In the more irregular and as a rule pernicious types of malarial infection which are met with in the autumn months a third variety of organism may be recognized, which has been specially studied by the Italian observers. The youngest forms of this parasite are small hyaline bodies about one-sixth the diameter of the red cell. At first they are quiescent, but later develop active amœboid movement. They are at this stage not unlike those of the tertian varieties; but the hyaline body is more signet ring-like, more highly refractile, and the central part often looks shaded, as if a more solid body were enclosed within a vacuole. As this form increases the amœboid movements are well seen. The pigment is in small amount, at first in the form of one or two very dark granules at the margin of the amœbæ, and the pigment never becomes so abundant as in the tertian or quartan forms. The

organism rarely occupies more than about one-third of the corpuscle, the stroma of which is never entirely decolorized. On the contrary, it often presents a curious brassy-green appearance, and looks shrunken or crumpled. The cycle of development of this form is rarely carried out entirely in the circulating blood, but the bodies with centrally placed pigment are not uncommon. The observations of the Italian observers seem to show conclusively that the segmentation takes place in the spleen and in the bone-marrow and internal organs. The length of its cycle of development has not been determined. Probably different groups mature at varying intervals of time, from twenty-four hours or less to forty-eight or more (Welch). The fever associated with this organism is characterized by irregularity, the paroxysms are not at definite periods, and the pyrexia may be more or less continuous, with remissions. This form is associated with the severer types of the malaria seen in late summer and autumn—the æstivo-autumnal fevers of Cuba, Italy, etc.

There are several other points of interest about the parasites. A corpuscle containing a half-grown organism may suddenly rupture; the hæmoglobin diffuses, and the pigmented parasite is set free. The parasite may break up into two or three portions, perhaps from pressure on the slide, and slight amœboid changes may be seen. In other instances, apparently from certain free extra-corpuscular organisms, the remarkable *flagellate* form develops itself. The pigment becomes more central, and the granules dance with great activity. Suddenly, long, thread-like processes extend from the body of the parasite and display remarkable movements, thrashing about over the corpuscle with extra-

ordinary rapidity. A flagellum may break off from the main body and move about independently among the corpuscles. While these flagellate bodies appear in both the tertian and quartan fevers they are very much more numerous in the irregular malaria. The significance of the flagellate form is still under discussion. By some it has been regarded as a degenerate form.

In the æstivo-autumnal, quotidian, or pernicious malarial fevers there is developed also a very striking body, to which much attention has been paid, viz., the "crescent" of Laveran. In any case of irregular malarial fever which has lasted a week or more these bodies are to be found. They are developed within the red blood-corpuscle, the margin of which may usually be seen on the concave surface of the crescent. The border is very sharply defined, the protoplasm uniform, homogenous, with coarse pigment granules, often in the form of rods, which are collected about the centre. Bodies similar in structure, but differing in form, being ovoid and rounded, are also met with; and the change of a crescent into an ovoid or rounded body can be traced, which, in turn, may in some instances be seen to project flagella or form a flagellated body similar to that derived from the extracorporeal organisms above referred to. Most authors say that both kinds of flagellate bodies do not develop unless the blood be exposed to the air, but an exposure of one or two minutes gives the best results. It would seem that they do not exist as flagellate forms in the circulation. (Osler, in Allbutt's *System of Medicine*.)

Pigmented Leucocytes. Typical pigmented leucocytes are very characteristic signs in malarial blood, and on

their presence alone the diagnosis must often rest: (1) In severe acute cases after the administration of much quinine; (2) in remittent malarial fevers; and (3) in chronic malarial fever and cachexia. They persist in the blood long after all traces of parasites have disappeared. The identification of free malarial pigment is usually hazardous, and the diagnosis of malaria should never be based on its presence alone (Ewing).

Inoculation Experiments. Malarial infection can be transmitted directly from man to man by subcutaneous or intravenous inoculation of malarial blood. This was shown first by Gerhardt in 1884. Later experiments, chiefly by Italian observers, have confirmed Gerhardt's investigations, and almost in every instance the variety of organism introduced has been reproduced. It has also been experimentally shown that the ague paroxysm is associated with the segmentation of enormous groups of intracorpuseular amœbæ, the symptoms being probably due, as Bacelli suggests, to toxins liberated during sporulation or to substances set free in the blood by the rapid destruction of a large number of its corpuscles. The period of incubation is from eleven to twelve days in the regular intermittents and from two to five days in the irregular autumnal fever.

Active phagocytosis goes on in all forms of malarial infection, but its true significance is still undetermined. That many parasites are devoured by the leucocytes, especially in the spleen, is certain. This apparently takes place during or after sporulation. But spontaneous recovery may also be due to the death of the plasmodia. It is not improbable, however, that the phagocytes contribute to the process of recovery, even if they are not the chief factors in it.

With regard to immunity, we know that one attack of malaria may linger a long time, and seems rather to favor than to prevent a new infection. There is, however, a natural susceptibility to the disease which is very variable. Different races of men especially seem to possess in variable degree the power of resistance to malarial infection. This is shown not only in a diminished tendency to contract the disease, but also in the form by which they are affected. For instance, the negroes in the Southern parts of the United States are much less liable to contract malaria than the whites; and Martin reports that the Europeans living in Sumatra are far more frequently and severely affected by malaria than the natives, who, if they are attacked at all, it is only with the simple intermittent tertian and quartan fevers.

The Action of Quinine on the Parasites. Laveran showed that a solution of 1 to 10,000 of quinine, run under the cover-glass, would check at once the movements of malarial organisms. As demonstrated by Marchiafava and Celli, however, a like effect is produced either by the water or by the salt solution in which the quinine is dissolved, and we meet with an almost insuperable difficulty in the study of the direct action of the drug upon the parasites themselves.

Many careful experiments have been made to determine the effect of quinine on the parasites circulating in the blood, and Romanowsky, Golgi and others have reported a diminution in the activity of the amœboid movements. Osler stated that, as a result of careful hourly examinations made in a series of cases with a view of ascertaining the direct influence of full doses of quinine, he was unable to make up his mind that any

particular change took place in the intracorpuseular tertian parasite while undergoing destruction by the specific.

The following points, nevertheless, about the action of quinine on the parasites seem to be well established : First, that under its use the intracorpuseular varieties, whether tertian, quartan, or æstivo-autumnal, rapidly disappear from the circulating blood; second, that quinine administered some hours before a paroxysm will not interrupt the cycle of their development, but will usually destroy the products of segmentation, and so check the succeeding paroxysm; third, that the crescentic and ovoid bodies which develop in æstivo-autumnal fevers are very slightly affected by the action of quinine.

Mixed Infection in Malarial Fever. It is now a well-known fact that along with a malarial infection there may exist another due to the typhoid bacillus, to one of the pyogenic cocci, or to other micro-organisms. Such mixed infection may make a complete diagnosis a very difficult matter.

Diagnosis. The diagnosis of malaria in all its forms has been greatly simplified by Laveran's discovery. This is not a matter of so much importance in the simple typical intermittents, but in the atypical forms of the disease, and especially in pernicious malaria, the symptoms of which are readily overlooked, serious errors in diagnosis may be made. Moreover, paroxysms of intermitting fever, which are common in other diseases, may be mistaken for those of malaria—such as occur in the early stages of tuberculosis, in ulcerative endocarditis, in suppuration associated with septicæmia or pyæmia, in pyelitis, etc. In all such

cases, and in cases of mixed malarial infection occurring in malarial regions, a careful blood examination enables a positive diagnosis to be made in a large majority.

Technique of Blood Examinations for Malaria. The finding of the parasite should not prevent us from seeking further in doubtful cases by means of the Widal reaction and blood cultures for other infections which may exist along with the malaria.

The parasites require a proper technique and a certain experience for their recognition. The fresh blood, when it can be obtained, should be examined, but if no bodies be found, stained preparations should always be later searched through; the drops may be taken either from the tip of the finger or from the lobe of the ear. It is important to have a perfectly clean cover-glass and slide, and to cleanse the skin thoroughly and to wipe it dry, so as to avoid dirt and perspiration. A very small drop should be taken, and care must be exercised that the cover-slips, when pressed against the blood-drop, do not touch the skin. The drop should be so small that the corpuscles are spread out in a uniform layer and are not in rolls when the cover-glass is laid upon the slide, for the intracorpuseular form cannot be well seen unless the blood-disk presents the flattened surface. For making permanent preparations the blood is collected upon cover-glasses in very thin films, which should be instantly dried. The blood-cells are fixed by immersion in equal parts of alcohol (95 per cent.) and ether for fifteen minutes, or by exposing for five minutes over a wide-mouthed bottle containing 25 per cent. solution of formalin, or by heating to 120° C. for ten minutes. Ewing advises

the alcohol and ether method. The preparations are stained with methylene-blue, or, if desired, with a double stain of methylene-blue and eosin. The preparation is covered with a mixture made of equal parts of a saturated alcoholic solution of eosin and water for one minute ; wash in water and dry in air. The preparation is then covered by a saturated watery solution of methylene-blue for a minute or two, washed in water, dried, mounted, and examined with the immersion lens. Thorough drying after the eosin staining makes the blue stain of the parasites sharper (Ewing).

In some cases of æstivo-autumnal fever the parasites are chiefly in the spleen, liver, and bone-marrow. The blood withdrawn directly from the spleen may show large numbers, although in the circulating blood they may be scanty. In these cases puncture of the spleen and examination of the blood withdrawn may render the diagnosis more certain, but in acute splenic tumor the procedure is not without risk. The finding of malarial parasites in the blood not only separates the intermittent, continued, and remittent malarial fevers from all other diseases in which similar fevers may occur, but the variety of parasites found influences the prognosis of the malarial infection. The number of parasites observed on examination also influences the prognosis to a certain degree, though too great weight should not be laid on this point, particularly as the result of a single examination. Whether there are any forms of malarial infection in which there are no plasmodia present in the circulating blood is a question for future determination. We know that in all severe seizures, if the blood is examined within twenty-four hours of the beginning of the paroxysms and before

much quinine is given, the plasmodia can readily be found, usually in considerable numbers. In some very mild initial paroxysms the plasmodia may be difficult to find. In æstivo-autumnal malaria, while quinine is being administered, there may be no organisms during the period between the second and fourth day, but on the fourth or fifth day the crescents almost always make their appearance, notwithstanding the use of quinine (Ewing).

Mode of Infection. It is generally acknowledged that the most common mode of infection in malaria is through the air. Whether the disease may be directly conveyed by water has been much disputed. Many favor the view, but experimental evidence is distinctly against it. Persons have been allowed to drink water from the Pontine marshes without ill effects, and in Bacelli's clinic at Rome experiments were made in thirty cases with water from malarial districts without a single positive result. Grassi could not produce the disease with dew from malarial regions or by allowing healthy men to drink blood from malarial patients. We may therefore assume that malarial infection is not produced, as a rule, by way of the intestines. Numerous experiments have shown, on the contrary, that the infection may be induced by subcutaneous inoculation. It is quite conceivable, therefore, that under natural conditions malarial infection may be produced by way of the skin, and possibly by the bites of insects. This is all the more probable, as certain varieties of mosquitoes, in malarial regions, have been found to be laden with the plasmodia. In another widespread disease produced by blood parasites—Texas fever in cattle—it has been shown that the amœbæ are con-

veyed by means of the cattle tick from animal to animal. The further the investigations have been pushed the closer becomes the connection between mosquitoes and malarial infection in man. So far as we know, a few varieties of mosquitoes and man are the only places where the malarial parasites develop, and Koch, following lines suggested by the work of others, has now shown that the fresh cases of infection with malaria occur only in warm weather when the parasites can develop in the mosquitoes. Koch's idea is that human beings having chronic malaria preserve in their blood the malarial parasites during the cool months. In the warm weather mosquitoes become infected, the parasites develop in them and are present in their poison sacs. These mosquitoes bite and infect fresh human cases through subcutaneous inoculation. He believes if we would treat all chronic malarial patients with quinine so as to prevent the development of the parasites and thus the infection of the new crop of mosquitoes, we would prevent most, at least, of human infection.

Blood parasites are extremely common in cold-blooded animals, fish, reptiles, and in birds. Birds appear to suffer from malarial infection similar to that in man, and the parasites found in the blood-corpuscles are closely allied to those of human malaria. But in birds infection cannot be produced by subcutaneous or intravenous inoculation with parasites from human blood, nor can infection be transmitted from birds to man. The blood parasites found in fish and reptiles, though similar to, are not identical with, those found in man, and they are not transmissible to man. This source of infection may, therefore, be excluded. Ex-

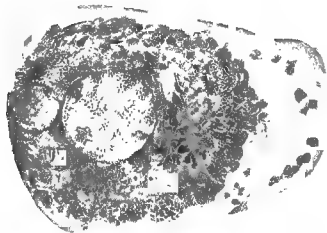
perience shows that the disease is not contagious, in the ordinary sense of the word, and that it is not directly transmitted from man to man.

AMOEBA COLI (*Amœba Dysenteriae* of Councilman and Lafleur; Dysenteric *Amœba*).

In 1875, Lösch, of St. Petersburg, gave the first accurate description of an amœboid organism which he found in the stools of a dysenteric patient, and to it he gave the name *amœba coli*. He claimed that this organism is the cause of dysentery, and he succeeded in producing a superficial ulceration of the large intestine in one of four dogs which had received rectal injections of the dysenteric stools. Lösch's observation has been confirmed by various researches in different countries.

Morphology. The amœba is a unicellular organism belonging to the class of rhizopoda of the protozoa, and consists of slightly differential masses of protoplasm, which, under favorable circumstances, exhibits spontaneous movements. In a state of rest the amœba assumes a spherical shape which appears discoid in the field of the microscope. It may generally be distinguished from the other cellular elements found in the feces by its pale greenish tint and by its stronger refraction of light. Its diameter varies within wide limits, 6μ to 35μ , more commonly between 12μ and 26μ . It is noteworthy that such differences in size are found, as a rule, in different cases of the disease, while the amœbæ in any individual case are nearly uniform in diameter. The body of a resting amœba has a well-defined, regular body, which, under ordinary conditions, appears as a thin, single, dark line.

PLATE II.



10

Figs. 1, 2, and 3 show three phases of the parasite of tertian fever. Fig. 1, ring form, showing beginning pigment formation. Fig. 2, full-grown parasite. Fig. 3, segmenting bodies. (WELCH and THAYER.)

Figs. 4, 5, and 6 show the parasite of quartan fever at different stages of growth. Fig. 4, moderately developed intracorporeal parasite. Fig. 5, large swollen extracorporeal form. Fig. 6, flagellate body. (WELCH and THAYER.)

Figs. 7, 8, and 9 illustrate the aestivo-autumnal parasite. Fig. 7, ring-like body, with a few pigmented granules. Fig. 8, crescent still in blood-corpuscle. Fig. 9, vacuolation of crescent. (WELCH and THAYER.)

Fig. 10. Amœba from section of intestine hardened in alcohol and stained with methylene blue. (COUNCILMAN and LAFLEUR.)

The body consists of two portions: the inner one, which is more or less granular and of a darker color, is known as the entoplasma; the outer one, which is homogeneous and of a lighter color, as the ectoplasma (see Plate II., Fig. 10). This division into two zones cannot always be made out, and is more evident in the motile than in the resting amœba.

The *entoplasma* constitutes the greater portion of the body of the amœba, being usually centrally situated, but occasionally slightly eccentric. In the smaller forms of amœbæ it is finely granular, and may show no other structure. In the larger forms it is more coarsely granular, and often contains clear, circular, and slightly oval spaces known as vacuoles. These are extremely variable in number and size.

The *ectoplasma* is quite homogeneous, forming a zone of variable thickness around the entoplasm. It has the appearance of finely ground glass of a distinctly pale green tint.

In most amœbæ a nucleus can be seen. Its detection is not always possible in fresh or motile amœbæ, but under certain conditions in the motionless or dead amœbæ the nucleus becomes evident, and it may be easily shown by appropriate staining reagents. It is situated eccentrically, at the edge of the entoplasm, and appears as a discoid body, about 6μ in diameter, with a sharp contour, which, though occasionally broken and irregular, is generally even; it may be distinguished from vacuoles of the same size by its higher refracting power. A nucleolus can seldom be observed, and in stained specimens only.

Foreign bodies are frequently seen in the amœbæ, especially red blood-cells. These are sometimes so

numerous that the whole body of the amœbæ is filled with them; they may be in a perfect state of preservation, or quite decolorized, or only recognizable by their outline. The amœba rarely contains leucocytes or fat-globules. Various forms of bacteria are more or less frequent inclusions, and black pigment granules and irregular brownish masses of pigment have been noted by some observers.

Biological Characters. The most striking and characteristic feature of the amœba is its motility. This may consist either in an alteration of its shape or in an actual change of place. Both of these phenomena are produced through the mechanism of pseudopodia. These latter are rounded, blunt, and homogeneous processes formed by the more or less gradual protrusion of a portion of the ectoplasm at some part of the periphery of the amœba. The motion is sometimes quite gradual and continuous, at others sudden and jerky. The progressive movement—that is, actual locomotion—is brought about by the protrusion of pseudopodia, and into these, when they have reached a certain size, the granular or vacuolated entoplasm, with its other contents, flows with a more rapid movement than that by which the pseudopodia themselves were formed. Locomotion is generally observed to take place in the direction of least resistance, a group of cellular elements or some detritus being sufficient to divert the course of the amœba. The amœboid movements are also influenced by various factors, particularly by variations of temperature. They are most active at the mean temperature of the human body, becoming less active as the temperature falls or rises above this mean. They become motionless in a tem-

perature lower than 75° F. The amœba does not take the stain of various coloring solutions until the movements cease, presumably on the death of the organism.

Practically nothing is known of the conditions of nutrition, respiration, and reproduction of the amœba, as no observations on these points are recorded.

Occurrence of Amœbæ in Man. Amœbæ were found in the stools by Kruse and Pasquale in forty out of fifty cases of the amœbic type of dysentery; by Kartulis in every case in nearly 500 observations; and by Councilman and Lafleur in thirteen out of fifteen cases; while in their remaining two cases the amœba was found post-mortem, either in the material scraped from the base of the intestinal ulcers or in sections of the latter. The number found is very variable. In some cases actively moving amœbæ are found in great numbers in every stool examined throughout the course of the illness, while in other cases they can be detected only in a long and careful search. As a general rule they are more numerous and more frequently present in the acute cases or in the earlier stages of the disease, or in the periods of exacerbations of chronic dysentery; and they disappear more or less gradually from the stools during convalescence. Occasionally the intestinal ulceration is latent, the motions being quite formed, with but small flakes of mucus adherent to them, in which no amœbæ may be found. In these cases the existence of dysentery is not suspected until an abscess of the liver occurs in which actively motile amœbæ are found, either by exploratory puncture or in the sputa if the abscess evacuates itself spontaneously through the bronchi.

Numerous investigations have demonstrated conclusively that amœbæ may be present in the feces of healthy persons. They have also been found in cases of chronic diarrhœa, cholera, intestinal tuberculosis, typhoid fever, hemorrhoids, and other diseases; chiefly in such as are accompanied by looseness of the bowels. Some of the cases cited as chronic enteritis or chronic diarrhœa were in all probability examples of the more chronic form of amœbic dysentery, but not all of them, of course. Temporary looseness of the bowels in otherwise healthy persons, either as the result of slight indisposition or of medication, seems to be a condition of the presence of amœbæ in the stools. Thus, Schulberg found these organisms in ten out of twenty loose stools produced by the administration of Carlsbad salts, and concluded that the amœba is a normal and harmless parasite of the intestines, the reason for its non-appearance in ordinary fecal evacuations being the solidity and acid reaction of the contents of the lower bowel, which soon destroy it. The question naturally arises whether more than one species of amœba is found in the human intestinal tract. So far no definite morphological differences have been found between the amœba occurring in the stools of healthy persons and that in patients suffering from dysentery; nor can any deductions be drawn from the attempts to cultivate the amœba, for no one yet has succeeded in producing pure cultures of it.

Pathogenesis. It is evident that, in the absence of artificially produced pure cultures of amœbæ, inoculation experiments must be made with material such as dysenteric stools or the contents of hepatic abscesses. In a few cases such material from hepatic abscesses which was found to contain no organisms other than

amœbæ, the inoculations have been made in three ways: by feeding animals with material containing the amœba, by inoculation of the small intestine after a preliminary laparotomy, and, finally, by rectal injections with or without suture of the anal orifice. The first method has always proved unsuccessful except when encysted forms were present. To the second method the objection has been raised that the manipulation of the intestines and the use of antiseptic solutions during the course of the operation are in themselves a source of irritation to the bowel, and in some cases have produced an enteritis. The third method is the simplest, and has given positive results in the hands of Löscher, Kruse, Pasquale and others.

The results of the last two observers were as follows: Dysenteric stools, or material from hepatic abscesses containing amœbæ, were injected into the rectum of various animals, with or without subsequent closure of the anus, for twenty-four or forty-eight hours. In some cases, chiefly those in which motionless amœbæ were injected, no abnormal result followed; in others, blood-tinged mucus, containing actively moving amœbæ, appeared in the evacuations from the second day or thereabouts, but the animals did not appear to be ill; in a third series, with evacuations of a like character, the animals wasted and died after a variable number of days. In both the second and third series of cases post-mortem examination showed pathological changes in the large intestine, proportionate, as a rule, to the severity of the symptoms. Of especial interest are the experiments made with material from liver abscesses which were proved to contain no other organism than the amœba. Three such cases are

recorded in cats, in all of which an experimental dysentery was produced. The lesions found are reddening and swelling of the intestinal mucosa, chiefly of the lower half of the large bowel, with here and there ecchymoses, small, superficial areas of necrosis, and shallow ulcerations. The mesenteric glands and the solitary lymphoid follicles are often swollen. In the blood-tinged mucus covering the mucous membranes amœbæ are found in greater or less numbers. Microscopical examination of sections of the intestine shows that the necrosis is limited, as a rule, to the mucosa, and that beneath it the submucosa is thickened and œdematous and its vessels engorged; there is also small-celled infiltration. Amœbæ are found in the borders of the ulcers, chiefly in the follicles of Lieberkühn; in the base of the ulcers they rarely penetrate more deeply than the upper layers of the submucosa. With the amœbæ are found many bacteria, chiefly streptococci.

From a comparison with the lesions of amœbic dysentery in man it will be seen that while the processes in man and in the cat are not identical, more especially as regards the depth and extent of the ulceration, yet in many points the resemblance is striking. A series of control experiments was undertaken, by the authors quoted, with amœbæ from the stools of healthy individuals and the straw-infusion amœba of Kartulis obtained in his culture experiments. In neither of these cases could an experimental dysentery be produced in any of the animals inoculated. They conclude that it is proper to designate the pathogenic amœba as the *amœba dysentericæ* (Councilman and Laffleur), and to retain the name *amœba coli* (Lösch) for the non-pathogenic amœba of the normal healthy intestine.

Concerning the source of the amœba and the mode of infection little can be positively stated. It is reasonable to suppose, however, that the mouth must be the usual path of infection, and that the amœba, in all probability, is taken with drinking water.

With regard to other organisms found in amœbic dysentery, a great number of bacteria of various kinds and some flagellated infusorial organisms are associated with the amœba in dysenteric stools.

CHAPTER XXXIX.

THE MICRO-ORGANISM OF SMALLPOX AND COWPOX.

No bacteria have been found in smallpox which seem to have any relation to the disease except as secondary infections. The same is true of vaccinia. In both the smallpox and vaccinia papules, vesicles, and pustules, L. Pfeiffer and others have constantly found small, homogeneous bodies in the epithelial cells surrounding the lesions. These little bodies are in the cell substance, not in the nucleus, and usually but one or two exist in any one cell. They are regularly missed in the skin when vaccination has failed, and also in similarly appearing papules and pustules in pyæmia, acne, etc. They apparently belong to the class of protozoa, and from their constant presence are believed to be the probable specific micro-organisms of both diseases. They are at first about double the size of the staphylococcus and increase to double that size (see Fig. 87, p. 651). Similar bodies have been noted in the blood. In a great many specimens of skin from cases of variola and vaccinia examined by Williams in the health department laboratories these bodies have never been entirely missed in the epithelial cells surrounding the lesions

The Connection Between Smallpox and Cowpox. The inoculation of the virus of smallpox into calves produces, when successful, in the first series moderate

redness and swelling at the point of inoculation, with some general disturbance. After the passage through several animals an affection exactly similar to cowpox occurs. The successful inoculation of the first series of cattle from smallpox is a matter of great difficulty, but so many experimenters have asserted that they have produced lesions similar to cowpox from smallpox that there seems no possibility of doubt that it has been done. In the laboratory we have failed in several attempts.

Experiments have demonstrated that children vaccinated with cowpox vaccine are not susceptible to inoculation with smallpox lymph, and also that those who have passed through smallpox cannot be inoculated successfully with cowpox vaccine. The mutual immunity conferred by inoculation with either, the similar appearance of the bodies in the cells about the vesicles of both, and the statements from reliable sources that smallpox virus has produced in cattle a disease indistinguishable from cowpox, leaves hardly any doubt that the two are due to the same micro-organism, which has become modified by transmission through cattle. Why such passage should produce a permanent change in the virulence of the organism is undoubtedly a difficult matter to explain, but we must remember that we know practically nothing about the life-processes of this form of micro-organisms, and changes once produced in them may tend to become fixed.

The Duration of the Immunity Conferred by Vaccination. The immunity caused by successful vaccination is not permanent, and varies in its duration in different individuals. Although it may give some protection from smallpox for ten or fifteen years, it is not well

to count on immunity for more than two years, and whenever we are liable to exposure it is well to be vaccinated. If it was unnecessary it will not be successful, while if it is successful we have reason to believe we were liable to at least a mild smallpox infection.

Protective Substances Present in the Serum of Animals After Successful Vaccination. It has been repeatedly shown that the blood-serum of a calf several weeks after successful vaccination possesses feeble protective properties, so that the injection of one to two litres of it into a susceptible calf would prevent a successful vaccination. A further and more convincing fact has been demonstrated in the laboratory by Huddleston—namely, that when equal parts of a serum taken from a calf, two weeks after successful vaccination, and of an active vaccine are mixed together and inoculated in a susceptible calf the vaccine fails “to take.” The serum of an unvaccinated calf has no deleterious effect whatever when mixed with the vaccine. Serum taken even several years after vaccination, if the case is still immune, will inhibit very distinctly the action of fresh vaccine virus.

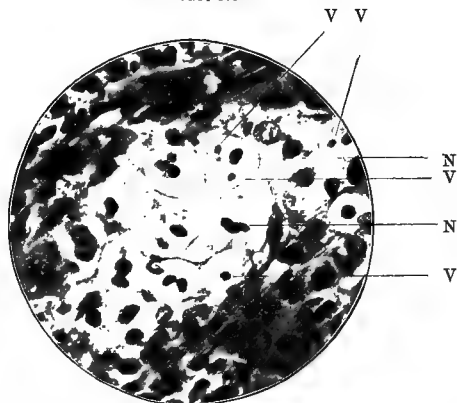
The Form of Vaccine Virus Used. Vaccination is now usually performed with calf virus, as this is easier to obtain, is just as reliable, and practically eliminates the slight possibility of the transference of syphilis which existed in human vaccine. With active virus a portion of skin only one-sixteenth of an inch in diameter is scratched with the needle and the virus rubbed in. If preferred it may be inserted by a puncture. The vaccine is now usually mixed with glycerin and water and placed in capillary tubes. So

prepared it is much more durable than when dried on ivory points or quills.

The Appearance of the "Vaccine Organism." Williams has described the appearances observed as follows :

"The epithelial cells of the skin in the infected areas of vaccinated calves contain characteristic bodies. These bodies are generally spheroidal, but may be quite irregular in outline. They vary in size from that of a

FIG. 87.



Epithelial cells of sebaceous gland of hair containing vaccine bodies.

V Homogeneous vaccine bodies. N. Granular nuclei. \times about 600 diam.

particle of nuclear chromatin to that of the nucleus itself, and are sometimes even larger. Usually they are about one-fourth the size of the nucleus. They are seen only in the body of the cell, never in the nucleus, and generally only one is seen in each cell, though there may be three or four. They take most nuclear stains rather more faintly than the nucleus, and are distinguished, moreover, by their homogeneous

appearance. With hæmatoxylin (Delafield's) and eosin the nucleus of the epithelial cells take an irregularly granular, dark purple stain, while the peculiar bodies are a fainter, homogeneous purple, and the cell-bodies pink'' (Fig. 87).

Horses, rabbits, and sheep were successively vaccinated with calf vaccine, but in none was the take anywhere as good as in calves, nor did it occur in every instance. Guinea-pigs and dogs failed to take in a few trials. The pulp and serum obtained from an epidemic of cowpox took feebly in calves in a moderate percentage of those inoculated. The characteristic vaccine bodies were found practically identical with those in vaccinia, except the bodies were a little larger and more irregular in outline.

The Preparation of Vaccine. For the following suggestions I am 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 exposed. It should be possible in this manner to charge at least from one to two hundred slips on each side of the slip from each child. The slips should be allowed a moment to dry and then placed in a sterilized box, in which, if kept in cold storage, they will probably remain efficient at least two or three weeks.

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 be vaccinated on the posterior abdomen and inside of the thighs easily 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 easily it may be vaccinated on the rump, each side of the spine; but the skin there is tougher, and the resulting virus, though efficient, is not so easily emulsified.

Vaccination. The calf should be cleaned thoroughly, including the feet and the tail, and the hair should be clipped from the end of the tail. The posterior abdomen and insides of the thighs are then 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 then made about one hundred scarifications, each from one-quarter to one-half of an inch on a side. 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 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 cotton and rubbed with the charged slips. One to two slips, depending on the amount of virus each slip contains, 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, remaining mass 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 from two to three times its weight of glycerin and water, equal parts, and this is done most effectively by passing the mixture between the rollers of a Doring mill. A watery pulp, especially if it is not to be used immediately, should have the smaller 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 to vaccinate the calf, because the use of fresh glycerinated pulp on a succession of calves leads to prompt degeneration of the vaccine and to the production of infected vesicles.

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 cleaner 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 licking the scarifications. If they are fed with milk the dust that would be imported with other food is avoided. 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, currycomb, mane brush, cord, halters, and with buckets, scrubbing brushes, and sponges. The operating-room should be well lighted and provided with a table and 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 passed 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 up on 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 grammes or centigrammes, 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, 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, and glycerin are the chemicals needed.

For issue for public vaccinations there are also needed packing-boxes, rubber bands, sheet wadding, needles, and wooden toothpicks (for removing the virus from the vials and rubbing it on the scarifications).

Yield. The material allowed from the five children should vaccinate at least five calves; it may easily vaccinate fifteen calves. Ten grammes 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 simply that the glycerin is not diluted sufficiently with water. We find one part of water to two of glycerin makes a good dilution.

Bacteria in Vaccine. It is impossible to prepare vaccine 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 was 126,360 in one loopful of vaccine virus, 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 contents 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 for a long time.

After two or three weeks the number of living bacteria is usually greatly diminished, but seldom totally destroyed. If we wait until the vaccine is surely sterile it is very apt to be also useless—that is, the vaccine bodies are dead also.

In a very large experience we have learned that the number of bacteria present has little to do with the resulting vaccination. The character of the vesicles in

the calves and the trial vaccinations in young children give a much more reliable basis for judging of the character of the virus than any bacteriological counts of colonies.

Pathogenic bacteria other than the practically non-virulent skin staphylococci are not found when animals are properly kept and vaccinated. The vaccine pulp and serum mixture is added to two and one-half to three and one-half times its bulk of a mixture consisting of two parts of chemically pure glycerin and one part of water.

Efficient vaccine should be inoculated in a portion of skin no more than one-eighth inch in diameter.

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.

CHAPTER XL.

RABIES (HYDROPHOBIA).

ALTHOUGH neither the nature of the micro-organism nor the nature of the poison of rabies has as yet been determined, it is here considered because of its special interest, and from the fact that it was the first infectious disease to which a curative, or, rather, preventive, method of inoculation was successfully applied.

Rabies is an acute disease of animals, dependent upon a specific virus, and communicated by inoculation to man. It is usually associated with an injury, such as the bite of a dog, and the inoculation of the broken surface with the saliva of an animal affected with the disease. This is the so-called rabies of the streets. Wolves, cats, foxes, and dogs; horses, cows, and deer may contract the disease; monkeys, rabbits, and guinea-pigs are all inoculable with it, as, indeed, are all warm-blooded animals. Rabies occurs in almost all parts of the world; it is most common in Russia, France, and Belgium; it is not infrequent in Austria and those parts of Germany bordering on Russia, and in England. It is comparatively rare in this country, although it occurs occasionally in various parts of the United States, also in Mexico and South America. Rabies is extremely rare in North Germany, Switzerland, Holland, and Denmark, owing to the wise provision that all dogs shall be muzzled; and in Australia it is unknown.

Etiology and Pathogenesis. The etiology and pathogenesis of rabies are still but imperfectly understood. The poison, whatever may be its nature, is usually contained in the saliva; and as early as the beginning of this century experimental rabies was produced in the dog by inoculation with the saliva of a hydrophobic patient. The bulk of the toxic material appears to be excreted in the saliva of the parotid gland, though a certain small quantity may be excreted by the other salivary glands, and also by the lachrymal glands, the pancreas, and the mammæ of rabid animals. The poison may also be found in the suprarenal bodies and in the fluid and substance of the cerebro-spinal nervous system, especially the medulla oblongata; it is found also in the peripheral nerves, though in much smaller quantity than in the central nervous system. It has not been found in the blood, the urine or the aqueous humor of the eye; it has been reported to have been found in the foetus.

That the disease is due to some form of organism which has the power of multiplying in the tissues and of producing a toxic substance, which appears to act specifically upon the central nervous system, cannot be doubted. As in other specific infectious diseases, the virus is transmitted directly from animal to animal through the medium of some fluid or secretion; it is now very generally recognized that the disease cannot arise anew, as was at one time assumed. In rabies, again, as in other infectious diseases, there is a period of incubation during which the poison appears to increase in quantity.

The certainty with which the disease may be produced and its severity have been found to be deter-

mined 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 hypothetical organism. It is a matter of common observation of hydrophobia in man that slight wounds of the skin, of the limbs, and of the back are often followed by the disease after an extremely long period of incubation; while in lacerated wounds of the tips 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 a similar abundance of nerves, the period of incubation is usually much shorter and the disease generally much more rapid. Experimental infection in animals is produced with the greatest certainty when the material from the rabic nerve-centre (the spinal cord or bulb) of a dog, or of a human being who dies of rabies, is injected into the dura mater of the brain. It may be produced almost as certainly when the injection is made into the anterior chamber of the eye or into the greater nerve-trunks. Intravenous injection is usually followed by positive results in small animals, but the larger animals do not succumb to this mode of inoculation. Subcutaneous inoculation in animals is uncertain, because the peripheral nerves are not always injured; but injection directly into a mass of muscle, especially into parts which are rich in nerves, almost invariably produces the disease. Absorption of the rabic poison, even from a healthy mucous surface, has been said to have taken place; and the conjunctiva, the nasal and genital mucous membranes, and the digestive tract have been noted as unabraded surfaces from which this has occurred. The

rapidity with which the virus is diffused through the body from the point of inoculation in the tissues seems to vary according to the location of the wound, but it is always comparatively slow. It has been found that rabbits, when etherized and then presented to a mad dog to be bitten on the fur, escape the disease in a very large proportion of cases, although the teeth may have passed well through the skin; if, on the other hand, the part presented to the rabid dog be shaved before it is bitten, the bitten animals contract rabies in a much larger proportion of cases. So in man, in many cases the rabic virus may be cleaned from the teeth by the clothing which covers the bitten part before they come in contact with the skin. From what has been said it is evident also that when the skin is thick and the nerves few a small quantity of virus may find its way into a wound, but not penetrate into the nerves, and thus the person bitten by a rabid animal may escape without any ill effects beyond those due to a lacerated wound. This will explain the fact that only about 16 per cent. of the cases bitten by rabid animals appear to contract hydrophobia.

Preventive Inoculation Against Rabies. The old treatment of rabies consisted simply in encouraging bleeding from the wound, or by 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 manifests itself after such heroic treatment if the wound be small; but when the wounds were numerous or extensive the mortality from it 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 therefore led to study the disease experimentally in animals, with the hope of finding some method of immunization or even cure through bacteriological methods; these investigations finally resulted in the discovery of methods of preventive inoculation applicable to man.

Immunization against rabies may be effected in several different ways. Pasteur's treatment is based upon the fact that rabic virus may be attenuated or intensified for any animal at will. He first observed that the tissues and fluids taken from rabid animals varied considerably in their virulence. Then he showed that the virus taken from similar positions—say the cerebro-spinal fluid—had always the same action in the same species; but that fluid taken from an animal of different species was weaker or stronger as the case might be. Thus the cerebro-spinal fluid of a series of dogs is of constant strength, and inoculations made from dog to dog regularly produce death from rabies, the animals passing through an incubation period fairly constant in length, and through a series of similar symptoms up to death at the same term. If, however, a series of monkeys be inoculated the virus gradually becomes attenuated, and this attenuation becomes more and more marked in successive inoculations until eventually, after the disease has run a longer and longer course in the successive animals, there comes a time at which the virus is no longer sufficiently active to cause death. If this attenuated fluid be now passed through a series of rabbits, dogs, or guinea-pigs it comes back to such a strength that it will kill, though slowly; then, however, its virulence gradually increases until the original intensity is

reached. If successive inoculations be made into rabbits with fluid, 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 one hundredth 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. Rabic virus appears also to become attenuated under certain conditions of temperature, and if it be subjected for about an hour to a temperature of 50° C. its activity is completely destroyed, or in half an hour if to a temperature of 60° C. 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 protected from heat, light, and air it retains its virulence for a long period. In his earlier experiments Pasteur selected a series of rabic poisons of different strengths, beginning with that obtained from the spinal cord of the monkey—from the very weak to the strongest that he could obtain in this animal—then passing through a similar series obtained during the process of exaltation of the virus by passage through the rabbit. By inoculating dogs subcutaneously with virus taken from a series commencing with the weakest taken from a monkey, and gradually working up to that obtained from the rabbit—from the earliest to the latest in the series—the animals become immune not only against subcutaneous injection but against subdural injection with fixed virus, and also against the bite of rabid dogs.

Such a method as this, however, had several disadvantages, and was not absolutely certain in its action, as only fifteen out of twenty dogs were completely protected. Pasteur, therefore, assisted by Chamberland and Roux, devised a more trustworthy and accurate method, in which he utilized the fact that the cord of a rabic animal when kept under certain conditions loses its virulence in fourteen days. A series of cords cut into short segments, which were held in series by the dura mater, were suspended in sterile glass flasks plugged with cotton stoppers, and containing a quantity of some hygroscopic material, such as caustic potash; and the whole was kept at a temperature of about 22° C. The cord when taken out at the end of the first twenty-four hours was found to be almost as active as the fresh untreated cord; that removed at the end of forty-eight hours was slightly less active than that removed twenty-four hours previously; and the diminution in virulence, though gradual, progressed regularly and surely until, as already noted, at the end of the fourteenth or fifteenth day the virus was inactive. An emulsion of the cord of the last day was made, and a certain quantity injected into a dog that had been bitten; this was followed by an injection of an emulsion of a thirteenth-day cord, and so on until the animal had been injected with a perfectly fresh and, therefore, extremely active cord, corresponding to the fixed virus. Animals treated in this way were now found to be absolutely protected, even against subdural inoculation with considerable quantities of the most virulent virus; and thus his protective inoculation against rabies became an accomplished fact. As it would be impossible, however, or very 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 two years, 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; very rarely, if ever, does an outbreak of the disease in man occur before an interval of at least fifteen days. The first symptoms usually appear in the fifth or sixth week, sometimes not until the third month; exceptionally the incubation period has lasted for a year. 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, are spread over five days are made in three days; then, on the fourteenth day, a fresh series of injections, or, rather, repetitions, is begun, which lasts until the twenty-first day. This is the "intensive method." In the technique of the treatment, which is the same in both methods, a small portion (about 1 cm.) of the desiccated cord is rubbed up thoroughly with about four or five times its bulk of bouillon until a complete emulsion is made; this, then, is injected by means of a syringe, holding several cubic centimetres, first on one side of the hypochondriac region and then on the other, the following day, and so on alternately, to avoid irritation. With the observance of thorough asepsis no local reaction to speak of takes place, nor are abscesses ever formed. 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, upward of 20,000 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 hands or whose clothes have been lacerated, so that the virus has passed into the wounds. Ordinarily, a certificate from a physician or a veterinarian that the animal was rabid is required before vaccination; but if the animal cannot be found or the wounds are severe vaccination is performed without it. Taking only the cases in which rabies has been confirmed in the animal by a veterinary surgeon, 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 statistics of Leblanc, from 1878 to 1883, out of 515 persons bitten in Paris 83 died of hydrophobia, a mortality of 16 per cent.; most authorities place the mortality at a much higher figure. Extensive bites on the face and head are considered to be particularly dangerous; the mortality of these is said to be at least 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 appears to be unavailable when symptoms of the disease have manifested themselves. Our results in the

New York Health Department have been very encouraging.

Other methods of immunization against rabies have been proposed by different investigators. But all of these methods have proved on trial to be unsatisfactory and unreliable, beside 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 from 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. This serum treatment of rabies is still in the experimental stage. We ourselves have had no experience with it, nor has it been adopted in Paris, or, so far as we know, in other places. It is quite possible that others will not obtain such good results as the authors of the treatment, or that it may not prove so efficacious in the treatment of man as it has been found to be in experimental work.

The Cauterization of Wounds Infected with the Virus of Rabies after an Interval of Twenty-four Hours. It is commonly believed that unless a cautery is used within an hour after infection by a suspected animal it is useless to apply it. This belief is held by physi-

cians in general, and also, apparently, so far as the literature seen by me indicates, by those familiar with rabies. For this reason physicians when applying a cautery later than an hour after infection do so largely as a matter of form, for its moral effect on the patient, and so the application is not thorough, and in consequence not effectual. There is no evidence to show that this is the case at all; no systematic investigations have been published, so far as we know, to prove the point one way or the other.

We know that the virus of rabies is not carried into the system by the blood, but through the nervous system. Dr. Follen Cabot carried out an extensive series of experiments in the laboratory upon guinea-pigs which showed: 1. That 91 per cent. of guinea-pigs can be prevented from developing rabies if the wounds be cauterized with chemically pure nitric acid at the end of twenty-four hours from the time of infection, probably a larger percentage if the cautery be used earlier. 2. That fuming nitric acid is more effectual than the actual cautery or pure nitrate of silver. 3. That some degree of benefit is derived from thoroughly opening and swabbing out an infected wound within twenty four hours from the time of infection when no cautery is used. I believe that he demonstrated that in cases in which the Pasteur treatment cannot be applied great benefit may be derived from the correct use of cauterization 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 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 indicates that physicians do not appreciate the value of thorough cauterization of the infected wounds.

But 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 where rabies prevails, hydrophobia would soon become an almost unknown disease. This fact has been amply demonstrated by the statistics of rabies in countries where such laws are now in force.



INDEX OF INFECTIOUS DISEASES AND BACTERIA FOUND IN THEM.

Abscesses. The bacteria most commonly found in acute abscesses are: *Staphylococcus pyogenes aureus*, 468, and *albus*, 470, and *streptococcus pyogenes*, 481-483. The following species are also occasionally met with: *Pneumococcus*, 509; *colon bacillus*, 449-453; *typhoid bacillus*, 410; *micrococcus tetragenus*, 472, and *influenza bacillus*, 324. In "cold abscesses" the *tubercle bacillus* is usually the only micro-organism present. Beside these bacteria other varieties may sometimes cause circumscribed suppurative processes.

Acne. Unna and Hodara (1894) obtained a bacillus from the contents of acne pustules which they believe to be the cause of true acne in man. *Staphylococci* are usually present in the pustules, and undoubtedly exert some influence in the production of the affection.

Actinomycosis. Due to the presence of the *actinomyces* or ray fungus, 618.

Alopecia. Although many dermatologists consider alopecia areata to be of neurotic origin, others incline to the belief that this affection is due to micro-organisms. Definite proof, however, is still wanting of the infectiousness of the disease, or of the causal relation of any specific micro-organism to it. Holborn (1895) described a micro-organism which he named *trichophyton radeus*, obtained it in pure culture and produced a similar affection in rabbits by inoculation, claiming that it was the cause of the disease in man.

Angina. When not diphtheritic the pyogenic cocci, 483, including the *pneumococcus*, are most frequently found in angina, also Vincent's bacillus, 354.

Anthrax. Due to the *bacillus anthracis*, 554.

Appendicitis. In thirty-two out of thirty-five cases of appendicitis bacteriologically examined by Hodenpyl (1893) the bacillus

coli communis, 451, 453, was the only micro-organism present. Streptococci and other varieties of bacteria from the intestines are also occasionally found.

Arthritis. The pneumococcus of Fraenkel, 509, has been frequently found in arthritis following pneumonia. The gonococcus of Neisser, 528, has been often met with in gonorrhœal arthritis. Streptococci, 483, and staphylococci, 469, have been obtained from the pus of the affected joints in suppurative arthritis following scarlet fever.

Beri-beri. Various species of bacteria have been found in the blood and tissues of persons affected with beri-beri, but none of these have been demonstrated to be the specific cause of the disease.

Bronchitis. From the sputa of patients with putrid bronchitis a spore-bearing bacillus has been obtained, the cultures of which gave off the characteristic odor of fetid bronchitis. Hitzig (1895) obtained two bacilli resembling the colon bacillus from a case of putrid bronchitis. In ordinary acute bronchitis the pneumococcus and streptococcus are most frequently found, but also small cocci like the gonococci in shape, and occasionally other bacteria, especially very small bacilli. In epidemics of influenza the influenza bacillus is frequently found.

Bronchopneumonia. The micro-organism most frequently met with in bronchopneumonia is the pneumococcus of Fraenkel, 507, 508, 511; next to this the streptococcus, 483; then Friedländer's bacillus, 458, and the staphylococcus—alone or in combination. At times the influenza bacillus, 325, is often found also. In pneumonia complicating typhoid fever the typhoid bacillus may be present in almost pure culture.

Bubo. The pus from an unopened inguinal bubo following chanroid is usually sterile, though it may sometimes contain the ordinary pus micrococci.

Bubonic Plague. Due to the presence of the bacillus pestis of Kitasato and Yersin, in the contents of the buboes and in the blood of infected animals and man, 607.

Carcinoma. No micro-organism has as yet been demonstrated to bear any causal relation to cancer. Some attribute the disease to protozoa.

Cerebro-spinal Meningitis. The micro-organism most frequently found in cerebro-spinal meningitis complicating other diseases is the pneumococcus, 510, 511, of Fraenkel; while in uncomplicated epidemic cases the diplococcus intracellularis meningitidis, 516, 519,

of Weichselbaum is usually found. *Streptococcus*, 483, *pyogenes* has also been met with in a certain number of cases, and occasionally the colon and typhoid bacilli and other species of bacteria.

Chalazion. Whether styne is a specific affection or due to mixed infection by the ordinary pus cocci is not known.

Chancroid. Ducrey (1890) discovered a bacillus, called by him *bacillus ulceris cancrisi*, which he obtained from the pus of soft chancres and buboes, and believed to be the cause of the disease, but he and others who have found it failed to cultivate it.

Cholera Asiatica. Due to the cholera spirillum, or Koch's "comma bacillus," 579.

Cholera Infantum. According to Booker and Jeffries, Baginsky and others cholera infantum is not due to a specific micro-organism, but to the action of the common putrefactive bacteria, such as the colon bacillus and the *proteus vulgaris* and other allied species, which, decomposing the food before it is digested, give rise to toxic products, which are then absorbed in the alimentary canal.

Cholera Nostras. Finkler and Prior (1884) obtained from the feces of patients with cholera nostras a spirillum which they believed to be the specific cause of this disease, but this has not been corroborated by experiment. It is more probable that cholera nostras, summer diarrhoea, and all this class of gastro-intestinal disorders are induced by the development of toxic products as the result of the ferment action of various species of bacteria, such as the colon, 452, and *proteus* groups.

Cholecystitis. The bacteria most commonly found are the colon bacillus, 453, and less often the typhoid bacillus. In the cases where typhoid infection is present the bacilli may remain in the gall-bladder for years.

Cholelithiasis. The colon, 453, and less often typhoid bacilli are met with. Typhoid bacilli have been found at operations for gallstones ten years after an attack of typhoid fever. (See Johns Hopkins Hospital Bulletin, 1899.)

Conjunctivitis. The specific infectious forms of conjunctivitis are undoubtedly due to the action of bacteria, as gonorrhoeal, 525, ophthalmia, and perhaps Egyptian catarrhal conjunctivitis (to the bacillus discovered by Koch and studied by Kartulis, Weeks and others), and diphtheritic conjunctivitis (to the Klebs-Löffler bacillus, 349, when associated with diphtheria, or perhaps to the xerosis, 348, bacillus). The non-infectious forms of conjunctivitis, however, are probably due, not to the action of specific micro-organisms, but

rather to some inflammation resulting from any cause aggravated by the presence of pyogenic micrococci.

Coryza. It is doubtful whether this affection is due to the action of any one specific micro-organism. Bacteria, however, play a part in keeping up the inflammation in acute and chronic nasal catarrh; and in *ozæna* the offensive odor of the nasal secretions seems to be due to the presence of certain bacteria, as Hajek's *B. foetidus ozænæ*.

Cystitis. It has been shown by recent investigations that cystitis is not caused by the mere presence of most varieties of bacteria in the bladder, except, perhaps, by a few varieties, such as the gonococcus, 528, provided it be healthy; but when the mucous membrane is injured by mechanical violence, or by the presence of a foreign body, cystitis is likely to result from the introduction of bacteria. The micro-organisms most frequently concerned in the development of chronic cystitis are the colon, 449, bacillus, the typhoid bacillus, the bacillus *aërogenes*, and varieties of the proteus bacillus. Among other bacteria which have been found in the bladder, and which may influence the production of chronic inflammation, are the tubercle bacillus, staphylococcus pyogenes aureus and allied species, the urobacillus, and the urobacillus liquefaciens septicus of Krogus.

Dengue. No specific micro-organism has been found in this disease which would seem to bear a causal relation to it.

Dental Caries. According to Miller (1894), who has made an exhaustive study of the bacteriology of dental caries, it is a mixed infection due to the presence of various micro-organisms in the pulp, cocci and bacilli being about equally frequent, with the occasional appearance of spiral forms. The typical pyogenic cocci are seldom present in the pus from the pulp, but various allied species are found which cause pus formation in mice. Putrefactive processes, also the result of bacterial action, greatly increase the action of the pulp cocci.

Diarrhœa. The action of bacteria in the production of diarrhœa has already been referred to under cholera infantum and cholera nostras. There is no reason to suppose that any particular micro-organism is the specific cause of this class of diseases, which are due probably to the toxins produced by various bacteria. Those which would seem to have most to do with the production of these troubles are bacilli of the colon and proteus groups.

Diphtheria. The Klebs-Löffler bacillus, 349, is now recognized to be the specific cause of diphtheria. Other bacteria, however, are always associated with this, producing more or less of mixed infec-

tion—viz., the streptococcus, 352, staphylococcus, 353, and pneumococcus, 353, mainly. Rather atypical pseudomembranous exudates are also produced occasionally by the pyogenic cocci, and fairly characteristic ones by the fusiform bacillus of Vincent, 354, and probably by other varieties.

Distemper in Dogs. According to Schantyr (1893) the so-called distemper in dogs includes three different infectious diseases: 1, abdominal typhoid, in which bacilli closely resembling those of typhoid fever in man are found in the blood and various organs; 2, dog-typhoid, in which bacilli are present which are readily cultivated and stain by Gram's method; and 3, genuine distemper, containing bacilli which stain by Gram's method, but which do not grow, or are difficult to grow, in culture media.

Dysentery. Trophic or amœbic dysentery is probably due, in the majority of cases, to the presence of the amœba coli found in the discharges. But this parasite has not been found in all forms of dysentery and in healthy stools. Among other bacteria found in the alvine discharges which may be concerned in the etiology of certain cases of dysentery are: the colon bacillus, the proteus bacillus, 542; the staphylococcus, the bacillus pyocyaneus, 539; the bacillus dysenterica liquefaciens, etc.

Eczema. Various species of bacteria, micrococci and bacilli have been obtained from cases of eczema seborrhœicum by different investigators, but none of these have been shown to be specific for the affection.

Empyema. The streptococcus pyogenes, 483, is the usual cause of purulent inflammation of the pleura, in which it is found in 60 per cent. of cases. Empyema complicating pneumonia is generally caused by the pneumococcus of Fraenkel, 511; and tubercular empyema is due, of course, to infection by the tubercle bacillus. The various micro-organisms are often found together in the same case, with one or the other predominating. In exceptional cases still other varieties of bacteria, as the typhoid bacillus, may be met with.

Endocarditis. Numerous varieties of bacteria have been found in pure culture or mixed in cases of ulcerative endocarditis, the most common being pneumococci, 509, 511, streptococci, 483, and staphylococci, 469; more rarely gonococci, 529, and other micrococci, and occasionally bacilli of several varieties, are found. Most probably the action of bacteria upon the endocardium is similar to that upon the bladder, and endocarditis, like cystitis, is not usually produced by them, unless some previous injury has been caused to

the valves, when their introduction then increases and maintains the inflammatory process.

Endometritis. The healthy uterine mucous membrane is usually sterile, but various species of bacteria have been observed in the secretions of the cervix uteri. In inflammations of the uterus not following abortion or child-birth the gonococcus is by far the most frequent micro-organism found. In inflammation following child-birth and operations the ordinary pus cocci and the colon bacillus are also frequently met with, as well as other varieties of bacteria.

Erysipelas. Due to infection by streptococcus, 483.

Fowl-cholera. Due to infection by bacillus cholerae gallinarum (Flügge), probably identical with the bacillus of rabbit septicæmia of Koch.

Furunculosis. Due to infection by the different pus cocci, and more especially to the staphylococcus pyogenes aureus.

Gangrene. Etiology not positively known, but probably due to the invasion of various parasitic and saprophytic bacteria into the tissues when their vital resistance has become lowered by malnutrition and pressure or by a poor blood-supply.

Gas-formation. The bacillus aërogenes capsulatus, 545, has been found either alone or along with pyogenic bacteria.

Glanders or Farcy. Due to infection by bacillus mallei, 600, 602.

Gonorrhœa. Due to "gonococcus," 528 (Neisser).

Hog-cholera. Due to infection by bacillus of hog-cholera (Salmon and Smith).

Hog-erysipelas or Swine-plague. Due to infection by bacillus of swine-plague (Salmon and Smith).

Hydrophobia. No micro-organism has as yet been discovered which is specific for this disease, 660.

Influenza. La Grippe. Due to infection by the bacillus of influenza, 324. Pneumococcus inflammations often show similar symptoms.

Influenza of Horses. Various micro-organisms, some resembling the pneumococcus and others the streptococcus in man, have been described and claimed to be the specific cause of this epidemic disease in horses.

Keratitis. According to Bach (1895) purulent keratitis is due to the invasion of the cornea by micro-organisms, the pyogenic cocci, pneumococci, etc., secondary to traumatism.

Leprosy. Due to the bacillus lepræ, 316

Lupus. Due to infection by the tubercle bacillus, 276.

Lymphangitis. Usually due to streptococcus pyogenes ; occasionally other organisms—viz., staphylococcus pyogenes aureus and albus and the colon bacillus, either alone or associated—take part in the production of this affection.

Malaria. Due to infection by the plasmodium malariae, 626.

Malignant Œdema. Due to infection by bacillus œdematis maligni.

Malignant Pustule. Due to anthrax bacillus.

Mastitis. The micro-organisms commonly found in mastitis are the ordinary pus cocci—staphylococcus and streptococcus. Diplococci corresponding to the gonococcus have also been observed in patients suffering at the same time from gonorrhœa.

Measles. All attempts to discover the etiology of measles, as of the other specific eruptive febrile diseases except, perhaps, smallpox, have thus far been futile.

Meningitis. (*See* Cerebro-spinal meningitis.)

Nephritis. The urine in acute infectious diseases, and also in cases of chronic nephritis, not infrequently contains various micro-organisms, which are also found in the blood or some other of the organs. Among the micro-organisms commonly found in nephritis secondary to general infection are: Streptococcus, staphylococcus, pneumococcus, bacillus coli communis, bacillus typhi abdominalis, etc.

Ophthalmia. There can be little doubt that most acute and some chronic inflammations of the eye are due to the presence of micro-organisms. As is well known, the gonococcus of Neisser is the cause of gonorrhœal ophthalmia, and, according to Fuchs, a considerable number of cases of so-called Egyptian ophthalmia are probably due to the same infective agent, while other cases are perhaps caused by the bacillus of Koch and Kartulis or by a combination of these two micro-organisms. Many bacteria when introduced into the eye give rise to inflammatory processes. The pneumococcus has been found by several investigators in cases of panophthalmia and other metastatic eye affections, sometimes alone or associated with the streptococcus and staphylococcus pyogenes. The bacillus pyocyaneus and bacillus coli communis have also been met with in these affections, the inflammation being undoubtedly due to the presence of the micro-organisms in the eye, which has been previously injured in some way.

Osteomyelitis and Periostitis. According to most authors the staphylococcus, 469, pyogenes aureus is considered to be the specific

cause of acute osteomyelitis ; but though present in many cases, alone or associated with other bacteria, this is not the only organism found in the affection. *Staphylococcus pyogenes albus*, *streptococcus pyogenes*, *pneumococcus*, and *bacillus typhosus* have also been found in osteomyelitis by various observers. This disease cannot, therefore, be regarded as a specific infection, but is rather a localized infectious process due to various micro-organisms. Chronic osteomyelitis and periostitis may also be considered in like manner as localized infections due to the tubercle bacillus.

Otitis Media. The micro-organisms most frequently found in the purulent discharges in recent cases of otitis media are : *Pneumococcus*, *streptococcus*, *staphylococcus pyogenes aureus* and *albus*, and Friedländer's bacillus. Occasionally found are : *Bacillus pyocyaneus*, *micrococcus tetragenus*, *bacillus coli communis*, and diphtheria bacillus, etc. These bacteria are undoubtedly responsible, directly or indirectly, for the inflammatory process and pus formation.

Ozæna. According to the investigations of Babes, Hajek and others the micro-organisms most constantly found in the nasal secretions of this affection are : Friedländer's bacillus, or a capsule bacillus closely resembling this, and the bacillus *ozænæ* of Hajek, though other species of bacteria are also often present.

Parotitis. Simple uncomplicated mumps is probably due to some specific micro-organism not as yet discovered, but the suppurative inflammation is undoubtedly caused by one or other of the ordinary pyogenic cocci. In parotitis occurring as a complication of other infectious diseases, as pneumonia and typhoid fever, the specific infective agents of these affections have been obtained in pure culture from the pus of the parotid abscess.

Pericarditis. Various micro-organisms have been found in the pericardial sac in pericarditis—the ordinary pus cocci, pneumococci, bacillus pyocyaneus, tubercle bacilli, etc.

Peritonitis. Among the bacteria found commonly in peritonitis are : The ordinary pyogenic micrococci, the colon bacillus, 449, 453, the pneumococcus, gonococcus, typhoid bacillus, tubercle bacillus, and proteus vulgaris. The pus cocci, especially streptococcus and the colon bacillus, appear to be the usual cause of the inflammatory process in puerperal peritonitis. In peritonitis following appendicitis and intestinal injuries the colon bacillus is always present either alone or associated with other bacteria.

Pleuritis. Levy (1895), from a résumé of the literature of the subject, arrives at the conclusion that the pneumococcus is the usual

ause of pleurisy in children and of metapneumonia pleurisy, but that in metastatic, pyogenic, pleuritic inflammation the streptococcus or staphylococcus are the common infective agents. Pleurisy due to streptococcus or staphylococcus infection is not in all cases attended with pus formation ; the exudate in a certain proportion of cases may remain serous.

In pleurisy occurring as a complication of typhoid fever the bacillus typhosus has been found in the exudate. Occasionally bacillus coli communis has been found. According to Flemming, about 41 per cent. of the fatal cases of pleurisy are due to tubercular infection.

Pleuropneumonia of Cattle. Due to infection by the pneumobacillus liquefaciens bovis of Arloing.

Pneumonia. Characteristic lobar pneumonia is due to infection by the pneumococcus, 507; 511; irregular cases are usually due to Friedländer's bacillus, streptococcus, staphylococcus, typhoid bacillus, and influenza bacillus, 325.

Puerperal Fever. Due usually to infection by streptococcus, 482, or colon bacillus, 453. In some fatal cases staphylococcus pyogenes aureus has also been found. Among other micro-organisms sometimes met with, and which in these cases may have been concerned in the production of the inflammatory process, are gonococcus and proteus vulgaris.

Purpura Hæmorrhagica. No micro-organism has been shown to be specific for this affection.

Pyæmia. (*See* Septicæmia.)

Pyelonephritis. According to Schmidt and Aschoff (1893), pyelonephritis or surgical kidney is an infectious process usually due to bacillus coli communis. (*See also* Nephritis.)

Pyosalpinx Zweifel (1892) has shown that a certain proportion of the cases of pyosalpinx, if not all of them, are due to the presence of the gonococcus, 528. In some cases the infectious agent is apparently streptococcus pyogenes or pneumococcus; but Zweifel believes that in the majority of cases in which the gonococcus is not found it is the infectious agent, its absence being due to the fact that it has died out in cases examined too late to find it.

Relapsing Fever. Due to infection by spirillum Obermeieri, 596.

Rheumatic Fever. The close analogy existing between true rheumatism and certain of the infectious diseases, such as gonorrhœa, scarlet fever, and septic processes in general, which are frequently

accompanied by arthritis and endocarditis, has led to the belief that acute rheumatism is an infectious disease. All investigations heretofore made, however, have failed to demonstrate the causal relation of the different bacilli and cocci isolated to the disease.

Rhinitis Fibrinosa. Pseudomembranous rhinitis is often associated with severe faucial diphtheria, and in these cases virulent Klebs-Löffler bacilli, 349, are present. The primary form of the affection, like conjunctivitis, usually runs a favorable course, and is due usually to the attenuated diphtheria bacillus; but here, too, occasionally virulent diphtheria bacilli are found in the fibrinous exudate. In such cases, of course, the nasal infection, however mild, may give rise to severe faucial or nasal diphtheria in others. In a few cases only pyogenic cocci have been found.

Rhinoscleroma. A localized infectious process due, apparently, to the presence of the bacillus of rhinoscleroma.

Rinderpest. The etiology of this acute exanthematous disease in cattle is still obscure. Recovery from an attack, however, produces marked immunity, and Koch has achieved considerable success in inoculating cattle against rinderpest.

Scarlet Fever. Streptococci are constantly present in large numbers in the pseudomembranous exudate of scarlatinal angina, 484, and not infrequently also in the blood and organs after death from scarlet fever. The presence of these streptococci in scarlet fever is probably due to the great increase in the streptococci usually existing in the throat secretions, and does not indicate any specific causal relation to the disease.

Septicæmia. General septicæmia in man is usually due to infection by one or other of the common pyogenic cocci—streptococcus, 481, 483, pyogenes or staphylococcus, 469, aureus and albus. Other micro-organisms which may sometimes be concerned in the production of septicæmia are the pneumococcus, 509, and colon bacillus. Septicæmia in cattle, deer, swine, rabbits, and fowls is due to infection by the bacillus of fowl-cholera or rabbit septicæmia specifically, but various other bacteria produce septicæmia also in rabbits, mice, swine, and fowls.

Stomatitis. Schimmelbusch, Lingard, Foote and others have described bacilli obtained by them from the necrotic tissues in cases of noma, but the etiology of the disease is by no means established.

Syphilis. The bacillus of Lustgarten, 309, is accepted by some to be probably the specific cause of the disease, but this is far from proven.

Tetanus. Due to infection by the tetanus bacillus, 388.

Texas Fever in Cattle. Due to infection by a blood parasite belonging to the protozoa, described by Smith under the name of *pyrosoma bigeminum*.

Tonsillitis. (*See Angina.*)

Trachoma. Various micrococci have been found in trachoma by different investigators and claimed by them to cause the affection, According to Fuchs and Hoor, trachoma is frequently, if not always, due to infection by the gonococcus.

Tuberculosis. All forms of tubercular infection in man and animals are due to the bacillus tuberculosis. The bacillus which causes tuberculosis in cattle, 299, and the one which produces it in fowls, 300, though closely resembling the tubercle bacillus in man, possess some slight differences.

Typhoid Fever. Due to infection by bacillus typhi abdominalis, 402.

Typhus Fever. The specific causative agent of this, under certain circumstances, extremely infectious disease has not yet been determined.

Varicella. No micro-organism has been demonstrated to bear any relation to the etiology of this affection.

Variola and Vaccinia. Probably due to protozoa, 651. The common pus cocci and various other micro-organisms are found in the characteristic pustular eruption; their presence is due to secondary infection of the pustules, and has nothing to do with the cause of the disease, 657

Whooping-cough. Considered by Koplik and others to be due to a small bacillus found in the nasal and bronchial secretions in cases of the disease, 613.

Wool-sorter's Disease. Due to anthrax bacillus.

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
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