EXPLANATION OF PLATE X.

Fig. 1.—Bacillus Tuberculosis in Sputum. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

Fig. 2.—Bacillus of Leprosy, Section of Skin Nodule. × 1,000. Photomicrograph from Bowhill's "Bacteriology" by permission.

Fig. 3.—Bacillus of Influenza in Bronchial Mucus. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

Fig. 4.—Bacillus of Diphtheria (Klebs-Loeffler). Blood-serum culture stained with Loeffler's solution of methylene blue. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

Fig. 5.—Bacillus of Diphtheria. Stained with Neisser's solution, showing bodies of bacilli in smear faint brown; points, dark blue. × 1,000. Photomicrograph from Park's "Bacteriology" by permission.

Fig. 6.—Pseudo-Diphtheria Bacillus, Small Type. × 1,000. Photomicrograph from Park's "Bacteriology" by permission.

Fig. 7.—Bacillus of Typhoid Fever, from Agar Culture. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

Fig. 8.—Bacillus of Typhoid Fever with Flagella. Agar culture. × 1,000. Photomicrograph from Bowhill's "Bacteriology" by permission.
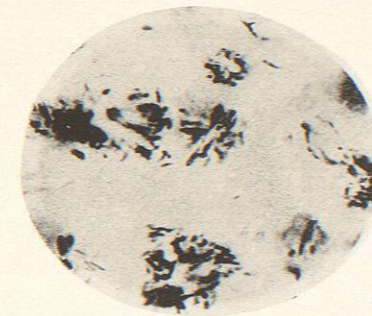
Pathogenic Bacteria.

I. Tubercle Bacilli in Sputum.
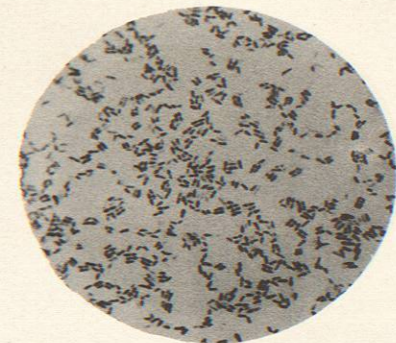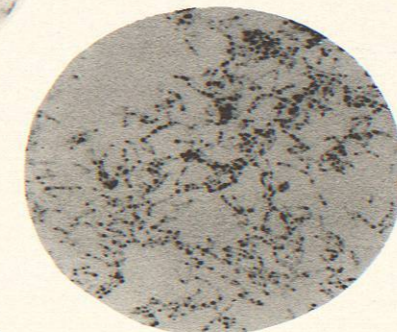
VI. Pseudo-diphtheria Bacillus. (Small Type).

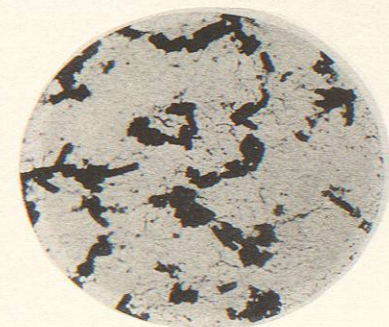IV. Diphtheria Bacillus. (Blood-serum Loeffler's Methylene-blue stain).

II. Leprosy Bacillus.

VII. Bacillus of Typhoid Fever.

V. Diphtheria Bacillus. (Neisser Stain).

III. Influenza Bacillus.

VIII. Bacillus of Typhoid Fever with Flagella.

Diphtheritic false membrane, analogous to human diphtheria, may be produced in animals by rubbing in diphtheria bacilli on the slightly abraded surface of mucous membranes of the trachea and conjunctiva of rabbits, of the throats of monkeys, and of the pharynx and larynx of pigeons and chickens. The process remains local. According to Loeffler, the best results are obtained by inoculation of the vaginal mucous membranes of guinea-pigs.

In man no experimental inoculations have been made, but in two involuntary laboratory experiments made in the New York City Health Department severe diphtheria was contracted by inadvertently sucking up virulent bouillon cultures of the diphtheria bacillus into the mouth.

Outside of the body diphtheria bacilli have been found upon articles used by diphtheria patients, as upon linen, brushes, toys, walls and floors of rooms, etc., and in the hair of nurses. The air (exclusive of momentary contamination through the coughing of patients) never contains the bacilli. They have also been found at times in the throat and nasal cavities as well as in the conjunctiva of healthy individuals, especially of those coming in contact with diphtheria patients. Out of three hundred and thirty healthy persons who had not been in contact, so far as known, with cases of diphtheria, Park and Beebe found virulent bacilli in eight only, two of whom later developed the disease. It is evident, therefore, that infection from diphtheria, as in other infectious diseases, requires not only the presence of virulent bacilli in the throat, but also an individual susceptibility at the time to the disease. Among the predisposing factors which may contribute to the production of diphtheria are the breathing of foul air and living in over-crowded and ill-ventilated rooms, poor food, and certain other affections, more especially catarrhal inflammations of the mucous membranes, but all depressing conditions in general favor the development of the disease.

The chief locations of the bacilli in diphtheria are on the surface of the pseudo-membranous inflammations of the fauces, larynx, and nasal cavities, but also occasionally in membranous affections of the skin, vagina, rectum, conjunctiva, nose, and ear (membranous rhinitis and otitis media). Occasionally they have been found in the blood and interior organs (spleen and kidneys).

Almost always the streptococcus pyogenes is associated with the diphtheria bacillus, with which it acts pathologically as a synergist. Regarding the importance of *mixed infection* in diphtheria, Bernheim has stated that the streptococcus products of decomposition favor the growth of the diphtheria bacilli and increase their virulence for production of toxin. Nevertheless, the diphtheria bacillus alone undoubtedly may produce all the clinical symptoms of sepsis.

Non-Virulent Diphtheria Bacilli.—There are sometimes found in inflamed throats as well as in healthy throats, either alone or associated with virulent diphtheria bacilli, micro-organisms which though morphologically and biologically identical with the Klebs-Loeffler bacillus, appear to be non-virulent—that is, in artificial culture media and with the usual animal tests they produce no appreciable diphtheria toxin. Between the bacilli which produce a great deal of toxin and those which seem to produce none at all we find all grades of virulence. These are probably attenuated varieties of the diphtheria bacillus which have lost their power of producing toxin (Roux and Yersin). Bacilli are also found which resemble the Klebs-Loeffler bacilli very closely except in toxin production, but differ also in some other respects. From varieties of this kind having been found in a number of cases of so-called *xerosis conjunctivæ* these bacilli are often designated as *xerosis bacilli*. They are usually much larger than diphtheria bacilli and have club-like extremities. They may be almost non-pathogenic for guinea-pigs, or they may kill. Animals are not protected by diphtheria antitoxin from the action of these bacilli. Whether they are derived from the original diphtheria stock is not known.

Pseudo-Diphtheria Bacilli.—Besides the typical bacilli which produce diphtheria toxin and those which do not, but which, so far as we can determine, are otherwise identical with the Loeffler bacillus, there are other bacilli found in positions similar to those in which diphtheria bacilli occur, and yet, though resembling these organisms in many particulars, differ from them in certain important characteristics. The variety most prevalent is rather short, plump, and more uniform in size and shape than the true Loeffler bacillus, and the great majority of them in culture show no polar granules when stained by the Neisser method, staining evenly throughout with Loeffler's alkaline methylene blue solution. Their colony growth on blood serum is very similar to that of the diphtheria bacilli, but they do not produce acid by the fermentation of glucose, and they never produce diphtheria toxin. These are properly called *pseudo-diphtheria bacilli.* When found in cultures from cases of suspected diphtheria they may lead to an incorrect diagnosis; and here the Neisser method of staining is of value, though the only absolute test of virulence is by inoculation of susceptible animals. (See Plate X., Fig. 6.)

Pseudo-Membranous 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 pseudo-membranous inflammations. The streptococcus, staphylococcus, and pneumococcus are the forms most often found in angina simulating diphtheria, but there are also others which, under suitable conditions, take an active part in producing this kind of inflammation. But the bacteria which occur in this so-called *false diphtheria* are all morphologically and culturally distinct from the Loeffler bacilli.

Susceptibility and Immunity.—It is now commonly recognized that an individual susceptibility, both general and local, to diphtheria is necessary to contract the disease. Age has long been known to be an important factor in the production of diphtheria, children within the first six months of life being but little susceptible, most so between the third and tenth years, while adults are comparatively immune. An apparent inherited susceptibility to the disease has also been observed. Two attacks of diphtheria have rarely been known to occur in the same individual within a short time. But to what this natural susceptibility or immunity is due is as yet only partially understood. As the result of animal experiments, however, it has recently been shown that an artificial immunity against diphtheria can be produced, at least for a considerable period, by the development, in the body, of substances antidotal to the diphtheria toxin.

Animals may be immunized against the diphtheria bacillus in various ways: By treatment first with slightly virulent and then with highly virulent cultures of the bacillus; by injection of small quantities of attenuated cultures or of toxin, and then with gradually increasing doses; by injection of the blood serum of animals immunized in one of the above ways against diphtheria. In the earlier experiments on immunization against diphtheria the names of Fraenkel, Wernicke, Aronson, Roux, and others are conspicuous; but to Behring and Kitasato belongs the credit of the fundamental discovery that the blood serum of an animal immunized for certain infectious diseases may be employed for protective inoculations, and that in larger quantity it may even exercise a curative influence after infection has occurred. This is one of the greatest discoveries in scientific medicine of recent years, and the practical results obtained in the treatment of diphtheria, at least, have justified all the expectations that were entertained regarding it. The mortality of this fatal malady among children has been reduced fifty per cent. or more in places where diphtheria was prevalent and where the treatment was continuously and uniformly employed. As to immunity, it stands to reason that a disease which can attack the same person more than once within a comparatively short time does not belong to the class of affections producing a permanent immunity after recovery. It is, however, well known that a certain temporary immunity is thus conferred, and the blood serum

of persons during convalescence from diphtheria has been found to possess immunizing properties. The protection afforded by artificial immunization, therefore, does not last usually more than three or four weeks, but this is usually sufficient to tide over the period of exposure to infection, and if necessary repeated immunizing injections of the antitoxic serum may be given. Regarding the curative injections, the earlier the remedy is administered the better; one gran being the effect produced—this effect being, indeed, one of immunity or protection against further infection or absorption by the system of the diphtheria toxin, rather than of neutralization of the poisons already absorbed.

Preparation of Diphtheria Antitoxin.—The principal steps in the preparation of diphtheria antitoxic serum are the production of toxin, the immunization of the horses, and the testing of the antitoxin obtained from them. The following is the method in brief now employed in the laboratories of the Health Department of New York City: The strongest diphtheria toxin possible is obtained by taking a very virulent bacillus and growing it under the conditions already described. The culture, after a week's growth, is removed, and having been tested for purity is rendered sterile by the addition of ten per cent. of a five-per-cent. solution of carbolic acid. This sterile culture is then filtered through ordinary sterile filter paper and stored in full bottles in a cold place until needed. Its strength is tested by giving a series of guinea-pigs carefully measured amounts injected subcutaneously; should kill a 250 gm. guinea-pig. The horses used for immunization should be young and absolutely healthy. A number of such animals are severally injected with an amount of toxin sufficient to kill 5,000 guinea-pigs of 250 gm. weight (about 20 c.c. of strong toxin), the point of injection being usually under the skin of the neck or behind the shoulder. After an interval of from three to four days, so soon as the febrile 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 administered. If antitoxin is not mixed with the toxin only one-tenth of the doses above mentioned is to be given. At the end of about two months, increasing doses of pure toxin having been injected every five to eight days, from ten to twenty times the original amount is administered. In about three months the antitoxic serum drawn from the horses should contain at least 300 antitoxin units, when tested, and the best of them from 800 to 1,000 units, in each cubic centimetre. Very few horses ever yield over 1,000 units, and none so far has given as much as 2,000 units per cubic centimetre. If every nine months an interval of three months' freedom from inoculations is given, the best horses continue to 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. It is run into large flasks through a sterile rubber tube, and then allowed to clot, the flasks having been previously placed in a slanting position. From these the serum is drawn off after four days by means of sterile glass and rubber tubing, and is stored in large bottles, small vials being filled as needed for use. Every possible precaution should, of course, be taken in the preparation of the serum to avoid contamination. An antiseptic may be added to the serum as a preservative, but it is not ordinarily necessary. Kept from access of air and light and in a cold place, it is fairly stable, deteriorating not more than forty per cent., and often much less, within a year. When stored in vials and kept as above, diphtheria antitoxin contains within ten per cent. of its original strength for at least two months.

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. This power is utilized in testing antitoxin. Guinea-pigs of about 250 gm. 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 animal dies earlier, less than 1 unit was added. An antitoxin unit has thus been defined as "ten times the amount of antitoxic serum required to protect a guinea-pig weighing 250 gm. from death, when ten times the fatal dose of toxin is mixed with the serum and the mixture injected subcutaneously into the animal."

The Use of Diphtheria Antitoxin in Treatment and Immunization.—For the injection a hypodermic syringe is employed, holding 10 to 12 c.c., which must be previously thoroughly sterilized with alcohol and a five-per-cent. solution of carbolic acid. The injection is made at some point on the anterior surface of the body, as the abdomen or thorax or outer surface of the thigh, where there is an abundance of subcutaneous cellular tissue. Before injection the skin should be carefully washed with alcohol or some disinfecting solution. The serum is rapidly absorbed, and it is better not to employ massage over the point of injection. For treatment of mild cases of diphtheria the dose is 1,500 antitoxin units, for moderate cases 2,000 units, and for severe cases 3,000 units. When no improvement follows in twelve hours the dose should be repeated; sometimes 6,000 units or more may be required in a single case. For immunization of children or adults who have been exposed to diphtheria the dose is from 300 to 500 units, according to age, to be repeated if necessary at the end of two or three weeks. In all cases it is better to use a small quantity of a high-grade serum than a large quantity of a low-grade preparation, as there is in the former instance less danger of rashes and other deleterious effects. The only untoward results to be feared in any case in which proper aseptic precautions are taken in the injection, are occasional rashes with perhaps some slight rise of temperature. In suspicious cases of any severity, particularly in croup, it is better to administer the remedy at once, making a culture at the same time for bacteriological diagnosis, than to delay treatment until a positive diagnosis has been made by bacteriological examination.

THE BACILLUS OF TETANUS (*Bacillus Tetani*).—Nicolaier in 1884 produced tetanus in mice and rabbits by subcutaneous inoculation of particles of garden earth, and showed that the disease was transmissible by inoculation from these animals to others. Carl and Rattone soon after this demonstrated the infectious nature of tetanus as it occurs in man. Finally, in 1889, Kitasato obtained the bacillus of tetanus in pure culture and described its biological characters.

Microscopical Appearances.—Slender rods with rounded ends, 0.3 to 0.5 μ in diameter by 2 to 4 μ in length, usually occurring singly, but often growing into long threads, especially in old cultures.

Spore Formation.—Forms rounded spores thicker than the cells, occupying one extremity of the rods and giving them the appearance of minute drumsticks. (See Plate XII., Fig. 3.)

Motility.—Motile, although not actively so in hanging-drop cultures with exclusion of air; numerous flagella are attached to the bodies of the bacilli. In the spore stage they are non-motile.

Staining Reactions.—Stains with the ordinary aniline dyes, and is not decolorized by Gram's solution. The spores may be demonstrated by double staining with Ziehl's method.

Biological Characters.—When freshly isolated from the animal body, this organism is strictly anaërobic; but by long cultivation at high temperatures it often becomes less sensitive to the presence of oxygen, this cultivation being facilitated by association with certain saprophytic bacteria. Carbone and Pessero have obtained from a

case of rheumatic tetanus in which there was no sign of injury in the bronchial mucous membranes virulent tetanus bacilli, which grew more luxuriantly under aërobic than anaërobic conditions; in pure cultures, however, they proved to be non-virulent. The bacillus tetani does not grow at temperatures below 14° C., though slowly from 20° to 24° C.; best at 37° C., when it rapidly forms spores. It develops in the ordinary nutrient gelatin and agar media of a slightly alkaline reaction. The addition of 1.5 per cent. glucose to the media causes the development to be more rapid and abundant. According to von Hibler, the less pathogenic the organism the more luxuriantly it grows on artificial culture media, and the more energetically it liquefies gelatin. In the animal body its growth is comparatively scanty, and it is usually associated with other bacteria, pure cultures being difficult to obtain. Kitasato's method, which is not always successful, however, consists in inoculating an agar tube with the tetanus material (pus from wounds), keeping this for twenty-four hours or more in the incubator at 37° C., and, after the spores have formed, heating it for about an hour at 80° C. to destroy the associated bacteria. The spores of bacillus tetani are able to survive this exposure, and anaërobic cultures are then made in the usual way, and the tetanus colonies isolated.

Growth on Gelatin.—On gelatin plates the colonies develop slowly, the middle portion being generally of a yellowish-brown color, with numerous threads radiating from the centre; the gelatin is liquefied. In old cultures the entire mass is made up of fine threads, the colonies presenting an appearance not unlike that of the common mould. In gelatin stab cultures the growth exhibits the appearance of a cloudy, linear mass with outgrowths radiating into the medium from all sides. Liquefaction takes place slowly, generally with the production of gas having an unpleasant empyreumatic odor.

Growth on Agar.—The colonies on agar are quite characteristic. To the naked eye they present the appearance of light, fleecy clouds; under a low-power microscope they resemble a tangled mass of threads. The extreme fineness of these threads enables the colonies of the tetanus bacillus to be distinguished from those of other anaërobic bacteria. In stab cultures on agar the growth resembles that of a miniature fir-tree.

Alkaline position is markedly clouded by the growth of the tetanus bacillus. It grows also in *acid culture media*, but itself produces no acid. *Milk* is not coagulated.

Vitality.—The spores of tetanus are very resistant to outside influences, retaining their vitality for months or years in a desiccated condition and not being destroyed in two and a half months when present in putrefying material. They withstand exposure to 80° C. for an hour, but are killed by a temperature of 100° C. in five minutes. They resist the action of five-per-cent. carbolic-acid solution for ten hours, but succumb when acted upon for fifteen hours. The addition of 0.5 per cent. hydrochloric acid to the carbolic solution enables it to kill the spores in two hours. In a solution containing 1 to 1,000 bichloride of mercury, five per cent. carbolic acid and 0.5 per cent. hydrochloric acid, the spores are destroyed in ten minutes.

Chemical Effects.—The tetanus bacillus produces gas in media containing sugar but no acid. It forms sulphuretted hydrogen abundantly and a little indol. It produces powerful toxins, which can be separated from the cultures by filtration. One one-hundredth of a milligram of an eight-day filtered bouillon culture is sufficient, as a rule, to kill a mouse. From this filtrate, however, the active toxin 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-gm. mouse in a dose of 0.00000005 gm. Reckoning according to the body weight of 75 kgm. or 150 pounds, it would require but 0.00023 gm., or 0.23 mgm., of this toxin to kill a man. Comparing this with other known poisons, the appalling strength of the tetanus toxin can

be readily appreciated. For instance, Calmette has found that dried cobra venom requires 0.25 mgm. to kill a rabbit of 4 kgm. weight, and it would thus require, at the same rate, 4.375 mgm. to kill a man of 150 pounds; the fatal dose of atropine for an adult is 130 mgm., of strychnine from 30 to 100 mgm., and of anhydrous prussic acid 54 mgm. The true composition of the tetanus toxin is unknown; it has been shown, however, that it is neither an alkaloid nor an albuminous body. The quantity of toxin produced varies, even when derived from one and the same culture, according to its age, composition, reaction, etc. It is extremely sensitive to the action of light, most chemical agents, and heat. It retains its strength best in the dry state.

Pathogenesis.—Man and almost all domestic animals are subject to tetanus. Among animals those most susceptible are horses, goats, guinea-pigs, and mice, less so rabbits and sheep; dogs, rats, pigeons, and chickens are almost immune. It is worthy of note that an amount of tetanus toxin sufficient to kill a hen would suffice to kill five hundred horses. A mere trace—only as much as remains clinging to a straight platinum needle—of an old culture is often enough to cause the death of mice and guinea-pigs.

On subcutaneous inoculation of virulent tetanus material mice and other susceptible animals show symptoms of typical tetanus in from one to three days. The parts first to be affected 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, then the tail, the other foot, the back and chest muscles on both sides, the fore legs, until finally there is a general tetanus of the entire body. In mild cases of infection, or when a dose too small to be fatal has been received, the tetanic spasm may be one-sided or remain confined to the muscles adjacent to the point of inoculation, and result in recovery. There may be no general increase of reflex excitability. In man and horses the local symptoms may be absent, but instead tonic spasms of special muscles; in man, of the muscles of the jaw, and in horses of the muscles of the jaw, neck, and tail. At the point of inoculation in test animals there may be on autopsy a hemorrhagic spot, but no changes here or in the interior organs other than this. A few bacilli may be detected locally with great difficulty, often none at all; apparently showing that the lesions produced are due, not to the multiplication of the bacilli in the living body, but to the absorption of the poison formed by them at the point of inoculation. It has been found that cultures freed from spores, and such as have been subjected to heat at 80° C., after sporulation and the toxins destroyed, can be injected into animals without producing tetanus. But if a culture of non-pathogenic organisms be injected simultaneously with the spores, or if there be an effusion of blood at the point of injection or a previous bruising of the tissues, the animal will surely die of tetanus. It would seem, therefore, from these experiments, 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, for here the infection may be considered as being probably always produced by the bacilli in their spore stage, and the conditions favoring a mixed infection are generally present.

Tetanus bacilli and their spores have been found widely distributed in garden earth, hay dust, floors of dwellings and hospitals, on splinters of wood, old nails, in the air, etc. They have apparently been observed more frequently in certain localities than in others, as in some parts of Long Island and New Jersey, but they are probably equally distributed everywhere. This bacillus is the chief etiological factor in the production, not only of trismus and traumatic tetanus, but also of all the various forms of tetanus—puerperal tetanus, tetanus neonatorum, and idiopathic and rheumatic tetanus.

Tetanus Antitoxin.—Behring and Kitasato were the first to show the possibility of immunizing animals against tetanus. Here the question of immunity against infec-