born of tuberculous parents, and persons living in poor hygienic conditions and depressing surroundings, as in prisons, asylums, and convents, and those suffering from exhausting diseases, more especially bronchial affections, diabetes, typhoid fever, etc., are more susceptible to tuberculosis than others not so situated or affected. Animal experiments, moreover, have shown that not only are there differences of susceptibility in various species, but also an individual susceptibility in the same species. The doctrine of individual susceptibility, therefore, is apparently founded on fact, although the reasons for it are only partially understood.

Immunization; Koch's Tuberculin.—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 agents that have been tried to protect animals against the action of the tubercle bacillus, the most important is Koch's tuberculin. Tuberculin contains all the products of the growth of the tubercle bacillus in nutrient bouillon and certain substances extracted from the bodies of the bacilli themselves; also the albuminoid and other materials originally contained in the bouillon which are unaffected by the growth of the bacilli. There are two preparations known respectively as the "old" and the "new" tuberculin or "tuberculin T. R."

Old tuberculin is prepared as follows: The tubercle bacillus is cultivated in peptone-glycerin-bouillon. At the end of from three to six weeks, according to the rapidity with which the culture grows, an abundant development takes place with the formation of a thick, dry, white crumpled layer, which finally covers the entire surface of the bouillon. (It was originally inoculated on the surface.) After development ceases, this layer breaks up and sinks to the bottom of the flask. Fully developed cultures, having been tested for purity by microscopical examination, are evaporated by boiling to one-tenth of their original bulk. The liquid is then filtered, and the crude tuberculin thus obtained contains forty to fifty per cent. of glycerin (the broth medium contained four to five per cent.), and keeps well, retaining its activity indefinitely. This substance when injected into tuberculous individuals affects the tuberculous process in a peculiar way. Very small doses produce a moderate increase of inflammation with slight elevation of temperature in tuberculous persons, while healthy individuals have neither fever nor marked local symptoms. The following is the method of treatment employed. After each injection, which should be large enough to cause a slight but not a great rise of temperature, a noticeable change in the tuberculous process results. The amount of tuberculin injection is constantly 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. Inasmuch as the bacilli themselves have not been directly affected by the treatment, when this is interrupted the tuberculous process is apt to progress (Koch).

Although Koch and some of his followers have apparently, from their reports, obtained satisfactory results in the treatment and immunization of man and animals with old tuberculin, the majority of investigators, after a short period of enthusiasm, have abandoned its use as very rarely beneficial, if not often injurious. Koch has, therefore, attempted to improve his method and has recommended a new preparation under the name of "*Tuberculin T R.*," or *new tuberculin*. The substances produced in the body by the old tuberculin neutralized the tuberculous toxins, according to Koch, but were not bactericidal. This he considered due to the nature of the envelope of the tubercle bacillus, which rendered it difficult to obtain the substance of the bacilli in soluble form without so altering it by heat or chemicals that it was useless for immunizing purposes. Immunity, he thought, was not produced in man for similar reasons, the bacilli never giving out sufficient toxin, perhaps, to bring about the production of curative substances. He therefore decided to grind up the dried bacilli and soak them in water, and thus obtain, if possible, without the aid of heat, a soluble extract of the cell substance of the bacilli, which he hoped would be immunizing. Buchner, by crushing under a great pressure tubercle bacilli mixed with sand and thus squeezing out their protoplasm, has obtained a similar substance, which he calls "tuberculoplasmin." The new tuberculin is thus a watery extract of the soluble portions of the unaltered tubercle bacilli. Owing to the method of preparation, it is evident that contamination is difficult to avoid, freedom from intact bacilli is uncertain, and the strength of the solution is variable. Twenty per cent. of glycerin is added to preserve the preparation. After three years' trial the results obtained with the new tuberculin have not proved better than those with the older preparation. The only form of tuberculosis which seems to be decidedly benefited by either the old or the new tuberculin is lupus. Relapses, however, are common.

The chief use to which tuberculin has been put is as an aid to the diagnosis of obscure cases of tuberculosis in cattle and man, and for this purpose it has proved to be of inestimable value. Cows are generally injected subcutaneously with 0.3 to 0.5 c.c. (diluted with water to 30 or 50 c.c.) of tuberculin and watched to see whether there is a rise of temperature of 1.5° to 3° C. in twelve to fifteen hours. Occasionally the reaction does not occur when the animals are in an advanced stage of the disease, but in such cases the test is not needed. The reaction never takes place, or one very much less marked occurs, in healthy animals, though small centres of infection are often difficult to locate later on autopsy. Latent tuberculosis is rarely if ever stimulated to renewed activity. It is important to note that an animal frequently requires an interval of a month to give a second positive reaction, if it has reacted typically on the first trial. In man it is, of course, much more difficult to form any opinion as to the reliability of the tuberculin test, from the fact that it cannot be controlled by post-mortem examinations; at any rate this test in man is at present not used extensively for diagnostic purposes.

Maragliano and others claim to have obtained with an antituberculous serum, prepared chiefly from horses, encouraging results; and Behring hopes soon to be able to make an antitoxic serum which will be curative and protective. But whether serum therapy is destined to solve the problem of the treatment of tuberculosis remains for the future to decide. Judging, however, from the progressive nature of the disease, there is not much ground to hope for the abundant development of curative substances in the blood of animals.

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 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 in adopting the necessary hygienic measures to stamp out tuberculosis entirely; but from the results which have already been obtained in reducing the mortality from this greatest scourge of the human race, we have reason to hope that in time it may be completely eradicated.

The Leprosy Bacillus (*Bacillus Lepræ*).—This organism, discovered by Hansen in 1879, is found chiefly in the interior of the peculiar round and oval cells met with in leprous tubercles. The bacilli have also been observed in the lymphatic glands, liver, spleen, and testicles, and in the thickened portions of nerves involved in the anæsthetic forms of the disease. According to some authorities they occur likewise in the blood. The bacilli lie in the leprous cells in great numbers, and also in the lymph spaces outside of these cells. They are not found in the epidermal layers of the skin, but, according to Babes, they may penetrate the hair follicles.

Microscopical Appearances.—The bacillus lepræ resembles the tubercle bacillus in form, but is 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. (See Plate X., Fig. 2.)

Motility.—Non-motile.

Staining Reactions.—The leprosy bacillus cannot be positively differentiated from the tubercle bacillus by staining reactions. It stains readily with the aniline colors and also by Gram's method. Although differing from the tubercle bacillus in the ease with which it takes up the ordinary aniline dyes, it behaves like the former in the manner in which it retains its color when subsequently treated with strong solutions of the mineral acids and alcohol. Inasmuch as leprosy and tuberculosis not infrequently occur together in the same person (according to Hansen and Looft tuberculosis being the cause of death in forty per cent. of the cases of leprosy), in making a differential diagnosis, all the various points must be considered, histological and pathological, and animal inoculations made, in addition to microscopical examination.

Biological Characters.—Attempts to cultivate the bacillus lepræ have frequently been made, but so far with only questionable results, as none of the cultures obtained has produced a similar disease when inoculated into animals. The etiological relation of this bacillus to leprosy is based, therefore, chiefly upon its constant presence in the leprous tissues. It has been shown by Spronk, however, that the blood serum of many lepers even in weak dilution gives the agglutinating reaction with cultures of the bacillus lepræ,—a fact which goes to prove that the organism cultivated is the true cause of the disease with which it is associated.

Pathogenesis.—Some investigators claim to have had positive results in inoculation experiments on animals with portions of leprous tubercles, excised for the purpose; but none has succeeded in producing the typical lesions of the disease as seen in man. Arning inoculated a condemned criminal in the Sandwich Islands with fresh leprous tubercles, his death occurring from leprosy five years later; but there is no conclusive evidence of the transmissibility of the disease in this way, as the man, according to Swift, had other opportunities for becoming infected.

It is generally assumed that infection takes place through the mucous membranes and through slight skin wounds. There is said to be no infection by way of the digestive tract. With regard to the question of direct inheritance from the mother to the unborn babe, there is considerable difference of opinion. Some cases of intra-uterine infection have been reported, but they are at least very rare. Leprosy bacilli are frequently present in the spermatic fluid and in the milk, but they have never been found in the ovaries. Most commonly they are met with in purulent nasal secretions (one hundred and twenty-eight out of one hundred and fifty-three cases examined by Sticker), and in the mucous membranes of the mouth, throat, etc.; but they have also been found in various other organs of the body, in the nerves, and in the blood. The widespread opinion, which was held before the discovery of the leprosy bacillus, that the disease was associated in some way with the eating of certain kinds of food, as salt fish, has now been generally abandoned. The negative results obtained from inoculation experiments, together with the fact that infection is not readily transmitted to persons exposed to the disease, have been explained by the assumption that the bacilli contained in the leprous tissue are mostly dead and nonvirulent; but it is much more probable that a special susceptibility to the disease, inherited or acquired, is requisite for its production.

The great similarity in many respects of leprosy to tuberculosis has recently been still more emphasized by the observations of Babes and Kalindero, who state that leprosy reacts, both localiy and generally, to an injection of tuberculin in the same manner as tuberculosis.

The Smegma Bacillus (*Bacillus Smegmatis*).—Found by Tavel and Matterstock in the smegma præputii, between the scrotum and thigh and between the labiæ; also in the cerumen and occasionally on the skin. The bacilli lie in clusters either in or between the epithelial cells, the rods being very similar, in size and form, to those of the tubercle bacilli. They stain with difficulty, and resist decolorization with acid when stained by the methods for staining the tubercle bacillus, but are decolorized when treated for one minute with absolute alcohol. This bacillus is most likely to be mistaken for the tubercle bacillus in the examination of urine.

Lustgarten's Bacillus of Syphilis.—This organism, which very closely resembles the tubercle bacillus and the smegma bacillus, was found by Lustgarten (1884) in the secretions of syphilitic ulcers and believed by him to be the specific cause of syphilis. Doutrelepont about the same time also observed a similar organism and came to a like conclusion.

Lustgarten's bacillus, though morphologically similar to the bacilli above mentioned, differs from them in staining reactions. It stains with equal difficulty as the tubercle bacillus, but is much less resistant to the action of acids; it is also more resistant, as a rule, to the decolorizing action of alcohol than is the smegma bacillus.

Numerous attempts have been made to cultivate this bacillus artificially 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, infection by inoculation frequently takes place, the tertiary lesions only being non-infectious.

Lustgarten's bacillus has been found in various syphilitic tissues, 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, however, of its causative relation to syphilis, and though 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, on the other hand, the long immunity in this disease (so different from that in any known bacterial affection) casts doubt not only upon the status of Lustgarten's bacillus, but also upon the bacterial nature of the micro-organisms producing it.

Baumgarten, who has searched in vain for Lustgarten's bacillus in uncomplicated visceral syphilomata, suggests that the bacilli found in such lesions were, perhaps, tubercle bacilli, and represented a mixed infection. Other micro-organisms have also been described and claimed to be the cause of syphilis, but none of these discoveries has been corroborated.

Recently (1899) Van Niessen has isolated from the blood and condylomata of syphilitics an organism which presents some of the characteristics of the diphtheria bacillus. This bacillus shows distinct club-like and branching forms and stains with the ordinary aniline dyes, and by Gram's method, but not with carbol fuchsin. It grows slowly on all culture media, developing whitish to yellowish colonies; and is said to be pathogenic for monkeys and pigs. Van Niessen lays particular stress upon the agglutinating reaction of his bacillus with the serum of syphilitic patients.

From this it appears that none of the organisms so far discovered has been conclusively proven to be the true cause of syphilis; and the position of Lustgarten's and Van Niessen's bacilli are at present too doubtful to make their detection of any diagnostic value.

The Influenza Bacillus (*Bacillus Influenzæ*).—Discovered by Pfeiffer and isolated in pure cultures (1891–92) from the purulent bronchial secretions of patients suffering from epidemic influenza. Pfeiffer's discovery has been fully confirmed by others, the results of whose researches give us reason to believe that this bacillus is the chief etiological factor in the production of influenza or "la grippe."

Microscopical Appearances.—Extremely small, moderately thick bacilli, about two or three times as long as broad, with rounded ends, occurring singly or in pairs,

but threads or chains of three or four elements are occasionally met with in cultures; often found in the interior of cells. (See Plate X., Fig. 3.)

Motility.—Non-motile.

Spore Formation.—Does not form spores.

Staining Reactions.—The influenza bacillus stains with difficulty with the ordinary aniline colors; best with dilute Ziehl's solution of carbol fuchsin or Loeffler's methylene blue solution, with heat. When faintly stained the two ends of the bacilli are somewhat more deeply stained than the middle portion. It does not usually stain with Gram's solution, though some investigators report such staining reaction.

Biological Characters.—Strictly aërobic; no growth occurs below 26° C., or above 43° C., or in the entire absence of oxygen; optimum temperature, 37° C. Grows on the surface of solid nutrient media containing hæmoglobin or pus cells, as *blood agar* or *blood serum*. At the end of eighteen to twenty-four hours on such culture media in the incubator very small, drop-like colonies are developed, which under a low magnification appear as shining, transparent, homogeneous masses; older cultures are sometimes colored yellowish brown in the centre. A characteristic feature of the growth of the influenza bacillus is that the colonies tend to remain separate, although when thickly sown in a film of moist blood upon nutrient agar they may occasionally become confluent. Spread out in a thin layer upon the surface of blood bouillon the growth develops as delicate white flakes. According to Grassberger a mixture of nutrient agar and defibrinated blood, which has been kept for one hour at 50° to 60° C., makes an especially good soil for their growth.

Vitality.—The influenza bacillus is very sensitive to desiccation; a pure culture diluted with water and dried is destroyed with certainty within twenty-four hours. In dried sputum vitality is retained for from twelve to twenty-four hours, according to the degree of drying. It does not grow, but soon dies in water. The thermal death point is 60° C. with five minutes' exposure. In bouillon cultures at 20° C. the bacilli remain alive for from a few days to two or three weeks.

Pathogenesis.—The bacillus of influenza, so far as is known, produces the disease by artificial infection only in monkeys and rabbits. From numerous experiments made in guinea-pigs, rats, mice, and pigeons these animals seem to be immune to influenza. When a small quantity of a twenty-four-hour-old culture on blood-agar is injected intravenously into rabbits, Pfeiffer found that a characteristic pathogenic effect was produced. Within one and one-half to two hours after the infection, the animals became very feeble, and suffered from dyspnœa, the temperature rising to 41° C. or more. At the end of five or six days they were able to sit up and move about again, and later they recovered. Larger doses caused death. When cultures were rubbed into the nasal mucous membranes of monkeys, these animals showed a febrile condition, lasting for a few days, but in no instance has Pfeiffer observed a multiplication of the bacilli introduced, the results being due to toxic products. Recently Cantani has shown that it is possible to produce an infection of influenza in rabbits when inoculated with small doses (.25 to .5 c.c.) of living bacilli, provided the point of least resistance is chosen, viz., the brain, the toxic products of the influenza bacillus acting most powerfully upon the central nervous system. The cell bodies of the bacilli seem to possess considerable pyogenic action.

It is possible that an *immunity* against the influenza poison lasting for a short period may be established after an attack. At least in three experiments made by Pfeiffer on monkeys, these animals, after recovering from an inoculation, seemed to be less susceptible to a second injection.

The influenza bacillus has not been found outside of the body. In patients suffering from influenza the bacilli are chiefly met with in the nasal and bronchial secretions, more especially in the characteristic light yellowish to green purulent sputum. The older the process the fewer bacilli will be found, and the more frequently will they be seen lying within the pus cells. At this time they stain less readily and present more irregular and swollen forms. Very often, perhaps almost invariably, the process invades portions of the lung tissue. In severe cases a kind of lobular pneumonia results, and is accompanied by symptoms almost identical with broncho-pneumonia. In fatal cases the bacilli have been found to have penetrated not only into the peribronchial tissue, but even to the surface of the pleura. The pleurisy which follows influenza, however, is usually a secondary infection, due to the streptococcus or pneumococcus. Ordinarily the disease runs an acute or subacute course, and not infrequently it is associated with a mixed infection of the pneumoccoccus or streptococcus. But sometimes a chronic condition may be produced depending upon the influenza bacillus; the bacilli remaining latent for a while and then becoming active again, with a resulting exacerbation of the disease. Phthisical patients are particularly susceptible to attacks of influenza. It would appear, therefore, that given proper climatic conditions, we have at all times the seeds of influenza present in sufficient numbers to start an epidemic.

The discovery of this bacillus enables us to explain many things previously unaccountable in the cause of epidemic influenza. We now know from the fact that the bacillus cannot exist for any considerable length of time in water or in dust, that the disease is not transmissible to great distances through these means. We also know that the infective material is contained chiefly in the catarrhal secretions. The occurrence of sporadic cases, or the sudden eruption of an epidemic in a locality from which the disease has been long absent, and where there has been no new importation of infection, may possibly be explained by the supposition, as already noted, that the influenza bacilli remain latent in the air passages of certain individuals for months at a time, and then become active under conditions favorable for their growth, when the infection may be communicated to others in close contact with them. The bacteriological diagnosis of influenza is of considerable importance for the identification of clinically doubtful cases, which from the symptoms may be mistaken for other diseases, such as bronchitis, pneumonia, or tuberculosis.

In acute uncomplicated cases the probable diagnosis can be frequently made by microscopical examination of stained preparations of the sputum, there being present enormous numbers of the small bacilli. In chronic cases or those of mixed infection the culture method must usually be employed if we wish to arrive at positive results. The bacillus of influenza is so well characterized by its morphological, staining, and cultural peculiarities that it may be distinguished from all other bacteria by an expert bacteriologist with sufficient certainty for diagnostic purposes. The only bacillus which at all closely resembles it is the *pseudo-influenza bacillus* found by Pfeiffer in three cases of broncho-pneumonia; and this is distinguished from the genuine influenza bacillus by its larger size and tendency to grow out, in cultures on blood agar, into long threads.

The Diphtheria Bacillus (*Bacillus Diphtheriæ; Klebs-Loeffler Bacillus*).—This bacillus was first observed by Klebs (1883) in diphtheritic false membrane. It was isolated in pure cultures and its pathogenic properties demonstrated by Loeffler in 1884. In 1887–88 further studies by Loeffler, Roux, and Yersin added to the proof of the dependence of diphtheria upon this bacillus. The results of these investigations have since been confirmed by a great number of combined clinical and bacteriological observations both in animals and man. All the conditions have been fulfilled for diphtheria which are necessary to the most vigorous proof of the causative relation of a given micro-organism to an infectious disease, viz., the constant presence of the organism in the lesions of the disease, the isolation of it in pure culture, the failure to produce the disease by any other bacteria, and the additional demonstration of the immunizing value of

the specific antitoxic substances developed in animals subjected to injections of diphtheria toxin. In view of these facts we are justified in concluding that all cases of true or primary diphtheria are due to the Klebs-Loeffler bacillus.

Microscopical Appearances.—Somewhat slender rods of variable size, 1 to 6 μ long and 0.5 to 1 μ broad, either straight or slightly curved, with rounded ends, occurring singly or in pairs. Irregular forms are very common, and indeed are characteristic of this bacillus. In the same culture and in unfavorable media great differences in form and dimensions occur; one or both ends may appear swollen, or the central portion may be thicker than the extremities, or the rod may consist of irregular spherical or ovoid segments. The rods sometimes lie in clusters alongside of one another in a characteristic manner, like a bundle of fagots. Threads with swollen ends and branching forms sometimes occur, but these are comparatively rare. (See Plate X., Fig. 4.)

Motility.—Non-motile.

Spore Formation.—Absent, but cultures retain their vitality for months.

Staining Reactions.—Stain readily with the ordinary aniline dyes and retain fairly well their color after staining by Gram's method. When Loeffler'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. Carbol fuchsin and gentian violet stain the bacilli too intensely, obscuring the structure of the organisms.

Neisser has recently described a double stain which brings out the metachromatic bodies of the diphtheria bacillus, and which he claims may be used as a method of differential diagnosis between the virulent and non-virulent diphtheria bacilli without the delay of inoculating animals. The cover-slip smear of diphtheria bacilli is placed for two or three seconds in a solution composed of alcohol (96 per cent.) 20 parts, methylene blue 1 part, acetic acid (glacial) 50 parts, and distilled water 950 parts, and then, after washing, in a second solution (for from three to five seconds) composed of Bismarck brown 1 part, and boiling distilled water 500 parts. By this method the bacilli are usually stained brown and at one or both ends a blue granule is seen; while the non-virulent bacilli ordinarily are not so stained. But sometimes the pseudo-diphtheria bacilli show the same dark bodies, and occasionally the virulent bacilli fail to take the Neisser stain. Neither this nor any other stain, therefore, can be depended upon to give positive information as to the virulence of the bacilli, the only certain way of obtaining a differential diagnosis between the pseudo- and true diphtheria bacilli being by animal inoculations with control injections of antitoxin. (See Plate X, Fig. 5.)

Biological Characters.—Aërobic and facultative anaërobic; grows best in the presence of oxygen, but also less readily without it. Development is good and abundant only at 37° C., the extremes being 20° and 41° C. It grows on all the ordinary culture media, glycerin agar being a favorable medium, though blood serum and ascitic fluid are still better. Loeffler's blood-serum mixture (see *Micro-Organisms: Technology*) is much used and is the best culture medium for diagnostic purposes in examining cultures from the throats of persons suspected of having diphtheria. The growth in gelatin at 22° to 24° C. is not characteristic, and is so scanty that it is seldom employed for the cultivation of the diphtheria bacillus. The gelatin is not liquefied.

Growth on Blood Serum.—On Loeffler's blood-serum mixture at the end of eight to twelve hours small colonies develop which appear as pearl gray, or more rarely yellowish gray, slightly elevated points. The borders are usually uneven. After forty-eight hours the colonies when separated may so increase in size that they are one-eighth of an inch in diameter; these lying close together become confluent and fuse into one mass, if the serum be moist. During the first twelve hours the colonies of the diphtheria bacilli are about equal in size to those of 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 blood serum is not liquefied.

Growth on Agar.—On one-per-cent. slightly alkaline, nutrient or glycerin agar the growth of the diphtheria bacillus is less certain and luxuriant than upon blood serum, but the appearance of the colonies when examined under a low-power lens is often more characteristic; the growth, however, is variable, and when obtained fresh from pseudo-membranes the colonies develop slowly or fail to develop at all. On *agar plates* the deep colonies are usually round or oval and as a rule present no extensions, but the surface colonies commonly from one and sometimes from both sides spread out an apron-like extension which exceeds in area the rest of the colony. These surface colonies are more or less coarsely granular in structure and usually have a dark centre. Some are almost translucent, others are thick and luxuriant with irregular borders shading off into a delicate lace-like fringe, though sometimes the margins are more even and the colonies are nearly circular. With a high-power lens the edges show sprouting bacilli, the colonies being gray or grayish white by reflected light and pure gray with olive tint by transmitted light. A mixture composed of two parts of a one and one-half 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. Nutrient plain or glycerin agar, with or without the addition of ascitic fluid, is the medium employed for the isolation of the diphtheria bacillus by plate methods from the original serum tube. The agar should be freshly melted and poured into the Petri dish for this purpose, and after it has hardened *streak cultures* from the colonies on blood serum are made upon this, the plates being left in the incubator at 37° C. for twelve hours.

Growth on Gelatin.—The growth on gelatin is much slower and more scanty than that on blood serum or agar, on account of the lower temperature at which it is used. Gelatin is not liquefied.

Growth in Bouillon.—In slightly alkaline or neutral bouillon the diphtheria bacillus grows in fine grains, which are deposited along the sides and on the bottom of the tube, leaving the broth nearly clear. Sometimes the bouillon may appear diffusely clouded to the naked eye, but when examined microscopically in the hanging drop the clumpy arrangement is readily observed. Frequently a whitish film forms over part of the surface, but in shaking this breaks up and slowly sinks to the bottom. This film is more apt to develop in cultures which have been long cultivated in bouillon. The reaction of the bouillon is subject to changes—the diphtheria bacillus in its growth causes a fermentation of the meat sugars with the production of acid; hence the bouillon becomes at first acid and subsequently alkaline, when the fermentable sugars have been decomposed, this latter change being favored by the admission of air.

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 a case of diphtheria may under certain circumstances be the means of conveying infection to previously healthy persons. The growth takes place better in raw than in boiled milk. The milk is not coagulated, remaining unchanged in appearance, but the cultures may retain their vitality for a long time.

On *potato* which is rendered alkaline a delicate coating develops.

Vitality.—Virulent diphtheria bacilli may persist in the throats of convalescents from diphtheria, after the disappearance of the false membrane, for weeks and months even. In 304 of 605 consecutive cases of diphtheria examined by Park and Beebe the bacilli were found to be no longer present within three days after the disappearance of the false 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, in 2 cases for nine weeks, and recently a case has been noted in which the virulent bacilli were present for six months. The practical importance of this fact is the evident necessity for the isolation of convalescents from diphtheria, whether showing clinical symptoms or not, until all the Klebs-Loeffler bacilli have disappeared from the throat.

In cultures kept in a cool, dark place, the bacilli retain their vitality for from six months to a year or more. In the incubator they are generally killed by desiccation in from one to three months; but even here, when the air is excluded, they remain alive in bouillon for a long time. They also retain their vitality for a considerable time in water and articles of food, etc.

The diphtheria bacillus possesses a considerable resistance to desiccation. Pure cultures in saturated silk threads at room temperature remain alive under favorable conditions for months. In dried diphtheritic exudate, even when pulverized, they retain their virulence for a long time. They are soon killed by moist heat at 60° C. Cold has comparatively little influence upon them, and even when dried they retain their virulence in winter for several months. Suspended in water and exposed to the action of direct sunlight the bacilli die in a few hours, but in agar and bouillon cultures they remain alive for six hours.

Chemical Effects.—The diphtheria bacilli produce gas and acids from carbohydrates, as from glucose present in ordinary nutrient bouillon. They also produce sulphuretted hydrogen and indol. In old cultures some nitrites are present, which with the indol give the nitroso-indol reaction on the addition of pure sulphuric acid. Pigment production is rare, though occasionally yellow to reddish species have been met with. Old bouillon cultures of the diphtheria bacillus filtered through porcelain produce the same symptoms as inoculations with the bacilli themselves. Particularly active toxins are obtained, according to von Dungern, by the addition of ascitic fluid to the bouillon. Sugar is to be avoided. Bouillon cultures as long as they are acid contain no toxins. A two-per-cent. peptone nutrient bouillon, having an alkalinity equal to about 8 c.c. of normal soda solution per litre above the neutral point to litmus, is a suitable medium for the development of toxin. Free access of air favors its production. The greatest accumulation of toxin in bouillon is after a growth of from five to ten days in the incubator at 35° to 37° C.

These poisons of diphtheria have been partially isolated. They are precipitated in part by alcohol, calcium phosphate, calcium chloride, and magnesium sulphate. The toxin has not yet been successfully analyzed, so that its chemical nature is unknown. It has many of the properties of proteid substances, but it is formed not only in albuminous culture media but also in those free from albumin. It is not a stable body, being totally destroyed by boiling for five minutes, and losing ninety-five per cent. of its strength when exposed to a temperature of 75° C. for some time. Temperatures under 60° C. alter it only very gradually. It is slowly decomposed when exposed to light and air, but kept in a cold, dark place it may be preserved almost indefinitely. According to Kossel diphtheria toxin is formed in the cell bodies of the bacilli and thence secreted. Ehrlich, who has recently investigated the subject of toxins, subdivides them, according to their degrees of toxicity, into protoxoids, syntoxoids, and epitoxoids.

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 scarcely at all for rats and mice. True diphtheria, however, as observed in man, is extremely rare among these animals, the so-called diphtheritic inflammations in them being due, as a rule, to other bacteria than the Klebs-Loeffler bacillus.

The virulence of pure cultures of the diphtheria bacillus from different sources, as measured by their toxin production, varies enormously. In general, severe cases of diphtheria yield strongly virulent cultures, and mild cases slightly virulent ones; but there are exceptions to this rule. One of the most virulent cultures so far known —culture No. 8, which is used not only by the New York Health Department Laboratory, but by many other laboratories in the United States and Europe, for the production of toxin—was obtained from an extremely mild case of diphtheria. Experimental and accidental attenuation of the diphtheria bacilli has often been observed. Roux and Yersin maintain that there is a uniform and gradual decrease in virulence of the bacilli found in the throats of convalescents from diphtheria, but this has not been confirmed by others, highly virulent bacilli having been repeatedly found in the throats of those recovering from the disease long after the disappearance of all clinical symptoms. 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, some diphtheria bacilli retain their virulence, when grown in artificial media, much longer than others. The passage of the bacilli through the bodies of susceptible animals does not increase their virulence to any appreciable extent, this being probably due to the fact that they multiply but little in the tissues.

The best guide for the virulence of a diphtheria bacillus is the toxicity of the filtrate of a culture of definite age, as shown by inoculation into guinea-pigs; for this purpose an alkaline broth culture of forty-eight hours' growth is used. 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. For an absolute test of specific virulence antitoxin must be used. A guinea-pig is injected subcutaneously with antitoxin, and then this and a control animal are injected with double the fatal dose of a broth culture of the bacilli to be tested. If the animal which received the antitoxin lives, while the control animal dies, it was surely a virulent diphtheria bacillus which killed by means of the toxin produced.

About twenty-four hours after the subcutaneous inoculation of a virulent culture of the diphtheria bacillus the animal becomes languid, has no appetite, its hair is rumpled, its nose cold and blue, and its respiration rough; the point of injection is infiltrated, sometimes also the surrounding tissues. Certain symptoms, however, exclusive of loss of weight, may be wanting. On autopsy there will be found at the seat of inoculation a grayish deposit surrounded by an area of congestion; the subcutaneous tissues for some distance around are œdematous; the adjacent lymphatics 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 or larger masses of necrotic cells, which are permeated with leucocytes. The heart and voluntary muscular fibres usually show degenerative changes. Occasionally there is fatty degeneration of the liver and kidneys. From the area surrounding the point of inoculation virulent bacilli may be obtained, but in the organs they are only occasionally found, unless an enormous number of bacilli have been injected. Paralyses, commencing generally in the posterior extremities and gradually extending to other portions of the body and causing death by cardiac paralysis or paralysis of the 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 produced.

Rabbits are much less susceptible to subcutaneous inoculation than guinea-pigs; white mice and rats are almost immune. On the other hand, cats, dogs, cows, and horses are susceptible, as are also young pigeons and chickens, and small birds.

EXPLANATION OF
PLATE X.