nevertheless, both in cholera patients and in inoculated animals, they have been met with in the organs—lungs, liver, kidneys, spleen, and occasionally the heart's blood. The more virulent the organism is, the more apt, apparently, is it to gain access to the interior organs.

Immunity.—Recovery from an attack of cholera produces a certain degree of immunity to the disease. Lazarus in 1892 observed that the blood serum of persons who had recently had cholera possessed the power of protecting guinea-pigs from infection by the cholera spirillum; while the serum of healthy persons or those affected with other diseases had no such effect. He attributed this to the presence, in the serum of convalescents from cholera, of antitoxic substances which neutralized the action of the toxins produced by the growth of the spirilla, in the same manner as the antitoxins of diphtheria and tetanus neutralize their respective toxins. Pfeiffer, on the other hand, maintained that this serum contained bactericidal substances which killed the spirilla so rapidly when injected into the animal that they were not able to produce their specific poisons, and that thus the animal was protected. It is now generally admitted that the serum is strongly bactericidal and feebly antitoxic.

These specific substances present in the blood of cholera-immune men and animals act only upon organisms similar to those with which they were originally infected—producing immobilization and agglutination of the bacilli. Pfeiffer, who first observed this peculiar reaction in cholera serum, has shown, however, that the specific relation existing between the antibacterial and protective substances produced during immunization and the bacteria employed to immunize the animals is not confined alone to cholera. This discovery has given us an apparently reliable means of distinguishing the cholera and typhoid bacilli especially from all other similar organisms, and the diseases which they produce from other infections which may be mistaken for them, which has proved to be of great practical value as an aid to clinical diagnosis.

There are two methods, known as Pfeiffer's and Gruber's reactions, whereby genuine cholera spirilla may be differentiated from other similar vibrios:

1. *Pfeiffer's reaction* is produced as follows: The blood serum of an animal rendered immune to cholera, by inoculation of attenuated or dead cholera cultures, is mixed with ordinary bouillon in the proportion of 1 to 100, and in 1 c.c. of this mixture a platinum loopful (about 2 mgm.) of the species under investigation is added, and this then injected into the peritoneal cavity of a guinea-pig weighing about 200 gm. Every five minutes some of the peritoneal effusion is removed by means of a capillary pipette and examined microscopically both stained and unstained. If it is the true comma bacillus, the bacilli will be observed to become at first non-motile, then agglutinated into clumps, and finally (in about twenty minutes) to become disintegrated and loosened. When the above phenomena are absent, the organism belongs to another species. A control experiment should be made with a known cholera culture to avoid possible error.

2. *Gruber's reaction* is founded upon this, but he deserves the credit of having determined the amount of dilution required to agglutinate and immobilize the cholera spirilla when mixed with cholera-immune serum for microscopical examination in the hanging drop, without injection into guinea-pigs, thus simplifying the method for practical use. For this purpose the blood serum of a person suffering from a case of suspected cholera, or of an animal immunized against the species to be investigated, is mixed with a pure cholera culture in the proportion of 1 to 50 and upward, and the mixture at once examined in the hanging drop. If the spirilla become immobilized and agglutinated into clumps within twenty or thirty minutes, then they are genuine cholera spirilla; if not, the result is negative.

Within the last few years Haffkine in India has succeeded in producing an artificial immunity against cholera infection in man by means of subcutaneous injections of dead cultures of the cholera spirillum; and Kolle has found that the blood serum of persons thus inoculated gave a reaction similar to that of persons who had recovered from cholera, showing bactericidal and agglutinative substances from the fifth day, but most distinctly on the twentieth day and for months after the protective inoculation.

Spirilla Resembling the Spirillum Choleræ Asiaticæ.—When Koch's comma bacillus was first discovered its properties seemed so characteristic that it was considered an easy matter to distinguish it from all other bacteria. Since then, however, more and more similar organisms have been met with by various investigators, until now they have ceased to be designated even by special names. The following are among the best-known species:

Spirillum Finkler and Prior (*Vibrio Proteus*).—This organism was obtained by Finkler and Prior from the dejections of patients with cholera nostras which had been allowed to stand for some days. It has since been found to bear no etiological relation to the disease, and is only of interest on account of its resemblance in some respects to the cholera spirillum.

It occurs as more or less curved rods, usually somewhat longer and thicker than the cholera spirilla and not so uniform in diameter. Involution forms are common in unfavorable culture media. It is actively motile, a single flagellum being attached to one end of the rods. It does not form spores.

It grows equally well, in the presence and absence of oxygen, on the usual culture media at room temperature. On *gelatin plates* small, white, punctiform colonies are developed at the end of twenty-four hours, which under a low power are seen to be finely granular and yellowish in color; liquefaction of the gelatin around the colonies progresses rapidly and is usually complete in forty-eight hours. Isolated colonies on the second day form cup-shaped depressions. In *stab cultures on gelatin* liquefaction proceeds much more rapidly than with the cholera spirillum, a stocking-shaped pouch appearing in two days, while the entire gelatin is liquefied in about a week; a whitish film forms on the surface. Upon *agar* a moist, shining layer covering the entire surface is quickly developed. *Blood serum* is rapidly liquefied. On *potato* at room temperature a shining, grayish-yellow layer is formed, soon spreading over the surface. The cholera spirillum, on the other hand, produces no growth on potato at room temperature.

The cultures of the spirillum Finkler-Prior give off a strong putrefactive odor; in media containing sugar they produce acid; they do not form indol, and they have a greater resistance to desiccation than the cholera spirilla. The absence of the agglutinative reaction with a dilution of the serum of an animal immunized to cholera is a valuable differential sign.

This organism is pathogenic for guinea-pigs when introduced into the stomach after previous injection of soda solution and tincture of opium, similar symptoms being produced, only somewhat less marked, as with the cholera spirillum. Although originally observed in the dejections of persons affected with cholera nostras, it probably has no relation to this disease, having been seldom found since under such conditions by subsequent observers.

Miller's Spirillum.—In 1884 Miller observed a curved bacillus in dental caries which, from its microscopical appearances in cultures and from animal experiments, has been thought to be identical with the Finkler-Prior spirillum. The *vibrio heliogenes* of Fischer and the *vibrio Lisbonensis* of Pestana, and other similar spirilla met with from time to time, are also probably identical.

Deneke's Cheese Spirillum (*Vibrio Tyrogenes*).—This organism was obtained by Deneke from old cheese, but has since been rarely observed. Morphologically and culturally it shows greater resemblance to Koch's comma bacillus than does the Finkler and Prior spirillum. It occurs in curved rods and long spiral filaments, the

diameter of the segments being uniform throughout. On the other hand, it is somewhat more slender than the comma bacillus and the spiral turns are closer together. In its power of liquefying gelatin it stands between the cholera spirillum and the vibrio proteus, and its other characters are also so intermediary between these two species that they are scarce worth describing. It is said to form a thin, yellowish coating upon the surface of gelatin and agar stab cultures, and not to give the indol reaction; but these characteristics are not constant. The chief means of differentiating it from the cholera spirillum is by the serum-reaction.

Spirillum Metschnikovi.—This spirillum was discovered by Gamaleïa in 1888 in the intestinal contents of fowls dying of an infectious disease common to certain parts of Southern Russia, and presenting symptoms like those of fowl cholera. It has since been found by Pfeiffer in the waters of the Spree and by Kutcher in those of the Lahn. In the affected animals it is almost always found in the intestines, but also in the blood, producing septicæmia. This interesting micro-organism cannot be morphologically distinguished from the cholera spirillum; it occurs as curved rods somewhat thicker, shorter, and often more decidedly bent than the comma bacillus. It liquefies gelatin, as a rule, much more rapidly than the cholera bacillus does, but this varies. It gives the nitroso-indol reaction without the addition of nitrites, and coagulates milk with acid reaction. It does not give the serum reaction with cholera-immune serum.

The spirillum Metschnikovi is characterized by its pathogenic action for chickens and pigeons; a minute quantity of a culture injected into the breast muscles of these animals causes their death with the local and general symptoms of fowl cholera, except that the contents of the intestines have more the appearance of cholera and the spleen is rather diminished than enlarged. In the blood and œdematous fluid about the necrotic point of inoculation, the organisms are present in large numbers. Gamaleïa has claimed that by passing the cholera spirillum of Koch through a series of pigeons, upon which this organism is said to act similarly to the vibrio Metschnikovi, by successive inoculations, its pathogenic power may be greatly increased, and that when sterilized cultures of this virulent variety of bacillus are injected into pigeons they become immune to the vibrio Metschnikovi, and *vice versa*. But Pfeiffer denies this—and the negative results obtained from the serum reaction with Metschnikoff's spirillum and cholera-immune serum show that the organisms are not identical.

The Spirillum of Relapsing Fever (*Spirochæte or Spirillum Obermeieri*).—First observed by Obermeier (1873) in the blood of a patient suffering from *febris recurrens*. Bacteriologically very little is known of this micro-organism. It occurs as long, slender, flexible, motile spirals or wavy filaments, with pointed ends, usually from 20 to 30 μ long. Flagella and spores have not been observed. Typically the organisms are found only in the blood and spleen, not in the secretions of patients with relapsing fever, and chiefly at the height of the disease, seldom or never during the intermissions. They stain readily with the ordinary aniline colors, especially with fuchsin and Loeffler's methylene blue solutions; they do not stain by Gram's method.

They have never been cultivated in artificial media. When preserved in blood serum and 0.5 per cent. solution of salt, they retain their vitality for a considerable time.

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 one-half days, but exhibit only the initial febrile attack; no relapse such as is characteristic of the disease in man occurs. Extirpation of the spleen renders the disease more dangerous for these animals. Infection may be transmitted by inoculation also from one monkey to another. Although so little is known of this organism from a bacteriological standpoint, the fact of its constant occurrence in relapsing fever and of the communicability of the disease from man to monkeys by inoculation of the blood gives us grounds for assuming that this is the cause of the affection.

The Glanders Bacillus (*Bacillus Mallei; Rotzbacillus; Bact. de la Morve*).—This bacillus was discovered by Loeffler and Schütz (1882) in the tissues of animals affected with glanders. It was isolated in pure culture by several bacteriologists, almost simultaneously, and was proved to be the cause of the disease with which it is associated.

Microscopical Appearances.—Small bacilli (2–3 μ long and 0.4 μ broad) with rounded or slightly pointed ends; they usually occur singly, but sometimes in pairs, and they rarely grow out to long filaments. Involution forms are common in old cultures. (See Plate XII., Fig. 4.)

Motility.—Non-motile.

Spore Formation.—Absent.

Staining Reactions.—Stains with difficulty with the ordinary aniline colors; does not stain by Gram's method. The bacilli often exhibit a granular appearance (metachromatic bodies) which are especially visible with Neisser's stain.

Biological Characters.—Aërobic and facultative anaërobic, growing both with and without oxygen, but best in the presence of oxygen and at brood temperature, though it develops slowly at 25° C.; does not grow at over 40° C. It may be cultivated on all the usual artificial media, but best on five-per-cent. glycerin agar.

On *glycerin agar* at the end of twenty-four to forty-eight hours it forms whitish, transparent colonies which in six or seven days may attain a diameter of 7 to 8 mm. On *blood serum* a moist, opaque, shiny layer of a yellowish or dirty-brown color is developed. The serum is not liquefied. On *potato* the growth is very characteristic. At the end of twenty-four to thirty-six hours at 37° C., a moist, yellow, transparent coating develops, becoming deeper in color and denser in consistence until it finally presents a reddish-brown color, and the surrounding surface of the potato acquires a greenish-yellow tint. The cultures often exhibit long, felt-like, interlaced filaments not unlike the threads of the bacillus anthracis, and finally club-like enlargements. In *bouillon* a diffuse clouding takes place, a tenacious, ropy sediment being ultimately formed. *Milk* is coagulated with the production of acid.

Vitality.—The resistance of cultures of the bacillus of glanders is not very great. They lose their virulence quickly by natural weakening as early as the fourth or fifth generation; therefore in order to retain virulence it is necessary after two or three generations of cultures to pass the virus through a susceptible animal. According to Bonome the glanders bacillus dies in ten days when exposed to a temperature of 25° C.; but other authorities find that it may live for three months under similar conditions. Exposed to heat the bacilli are killed at 80° C. in five minutes, and at 100° C. in three minutes.

Corrosive sublimate solution (1 to 1,000) destroys the bacilli in fifteen minutes, and five-per cent. carbolic acid in one hour. The virulence is quickly lost in distilled water (six days); it is not destroyed by putrefaction.

Pathogenesis.—Among domestic animals, horses, asses, cats, dogs, goats, sheep are the most susceptible; less so pigs. Cattle and birds are immune. Among test animals, the field mouse, wood mouse, and guinea-pig are the most susceptible, the rabbit being less so, while white mice and house mice are comparatively immune. When pure cultures of the bacillus mallei are injected into horses and other susceptible animals true glanders is produced. The disease in the horse is characterized by the formation of ulcers upon the nasal mucous membrane. These ulcers have irregular, thickened margins and secrete a thin, virulent mucus; the submaxillary glands become enlarged and form a tumor; other lymphatic glands also become inflamed, and some of them suppurate and open externally, leaving deep ulcers; the lungs are finally involved and the breathing becomes rapid and irregular. In farcy, which is a more chronic form of the disease, circumscribed swellings appear in different parts of the

body, especially where the skin is thinnest, which suppurate and leave angry-looking ulcers with abundant purulent disharge. Pure cultures can be obtained from the interior of the suppurating nodules and glands which have not yet opened to the surface; but the discharge from the nostrils or from an open ulcer contains comparatively few bacilli, and these are associated with so many other bacteria which grow more readily than the glanders bacilli on culture media that it is difficult to obtain pure cultures in this way by the plate method. Here test animals are useful.

In guinea-pigs subcutaneous injections are followed in three or four days by swelling at the point of inoculation, and a tumor with caseous contents soon develops, then ulceration of the skin takes place. The lymphatic glands become inflamed, and in from two to three weeks symptoms of general infection appear. In male animals orchitis and epididymitis are present, while the internal organs (lungs, kidneys, spleen, and liver) are generally the seat of characteristic nodular formations. From these pure cultures may be obtained. The specific ulcers produced upon the nasal mucous membranes of the horse are rarely present in guinea-pigs. The process is often prolonged, and the animals may live from six to eight weeks after inoculation; or it remains localized in the skin. Intraperitoneal injection of guinea-pigs is usually followed by death in from eight to ten days, and in males the testicles are invariably affected. In female animals the disease may be communicated to the fœtus.

The bacillus of glanders has never been found outside of the animal body nor in healthy individuals. The disease occurs as a natural infection only in horses and asses, but it may be communicated to man by contact with affected animals, and usually by inoculation through wounds of the skin or mucous membranes. In man, where the virus enters, a local swelling appears, which spreads rapidly, accompanied by suppuration and cording of the neighboring lymphatics. Multiple abscesses are formed in the skin, muscle, and internal organs, and there are often suppurative changes in the joints, the disease at this stage resembling pyæmia. Characteristic glanders nodules appear in the mucous membranes, particularly of the nose, which soon disintegrate, forming ulcers. The disease not infrequently terminates fatally, death resulting from general infection carried by means of the lymph circulation.

It is often difficult to demonstrate microscopically the presence of the glanders bacillus in the nodules which have undergone purulent degeneration, or in the discharge from the nostrils, ulcers and glands. Strauss has proposed the following rapid method of diagnosis by inoculation of test animals: Some of the suspected material or culture is introduced into the peritoneal cavity of a male guinea-pig, making the inoculation directly in the middle line of the abdomen, to avoid introduction into the vesiculæ seminalis. If it is a case of glanders, the testicles begin to swell within thirty to forty-eight hours, and the skin over them becomes hyperæmic, shiny, and finally degenerates and shows evidences of pus formation. The diagnostic symptom is the tumefaction of the testicles.

The diagnosis of glanders in horses, in which the clinical symptoms of the disease may be obscure, as in chronic or subacute cases, may often be made by the use of *mallein*. Mallein consists of the filtered products of the glanders bacillus—albuminous compounds bearing a similar relation to glanders that Koch's old tuberculin bears to tuberculosis—prepared by evaporating a six-weeks'-old culture in five per cent. glycerin nutrient veal broth to ten per cent. of its original bulk. The dose of mallein is about 1 c.c. subcutaneously injected, which usually gives good reactions. An injection of mallein under the skin of a healthy horse has no effect or at most produces a slight local swelling and rise of temperature. Following an injection of mallein into a glandered horse two reactions are produced: a large and painful swelling at the point of inoculation and a rise of temperature to 104° or even 106° F. The rise of temperature, however, should not be taken alone as conclusively indicating glanders; it must be considered in connection with the local swelling and the general condition of the animal which is profoundly affected by the injection. The practical value of this test has been demonstrated by numerous experiments by veterinarians. No ill effects have been found to result from the injection of mallein in healthy horses. On the contrary, not only production of immunity, but some cures have been reported from its use.

The Bacillus of Bubonic Plague (*Bacillus Pestis Bubonicæ*).—This organism was discovered by Kitasato and Yersin, independently, during an epidemic of the bubonic plague at Hong-Kong, China, in 1894. This disease, like anthrax and leprosy, has a long historical record behind it. It is probably the disease which under the names of "Black Death" or "The Great Plague" decimated the population of Europe in the Middle Ages. The distribution of plague at the present time is fortunately a somewhat limited one, namely, a definite area in Asia known as the "Plague Belt," extending from Mesopotamia, as a sort of focus, northward to the Caspian Sea, westward to the Red Sea, southward as far as Central India, and eastward to the China Sea. The bacteriology of plague is almost the latest contribution to the science.

Microscopical Appearances.—Short rods, with rounded ends, about twice as long as broad, occurring singly, in pairs, or in short chains (especially in bouillon cultures), and often surrounded by a capsule. Involution forms are common. (See Plate XII., Figs. 7 and 8.)

Motility.—Non-motile, possessing no flagella; though Kitasato claims that it has very sluggish, scarcely perceptible movements, and Gordon states that by a special method of staining (Van Ermengen's method) he found polar flagella.

Spore Formation.—Absent.

Staining Reactions.—Stains with the ordinary aniline dyes, but in preparations made from pure cultures the characteristic bipolar staining, which is observed in preparations from blood and pus, is not readily obtained. Does not stain by Gram's method.

Biological Characters.—Strongly aërobic, growth being inhibited in the absence of oxygen. Develops on the usual culture media, but best on blood serum at 37° C.; also fairly well at room temperature.

On *gelatin plates* small, darkly defined granular colonies of a grayish-yellow to greenish color develop; the gelatin is not liquefied. In *gelatin stab cultures* it grows slowly on the surface and along the track of the needle. On *glycerin agar* it grows rapidly, forming a moist, grayish-white coating on the surface. On *blood serum* in the incubator, at the end of twenty-four to forty-eight hours, white, moist, transparent, and iridescent colonies are formed. *Bouillon* becomes diffusely clouded, but if inoculated with a cohesive mass of bacteria from an agar culture the bacilli develop as a granular or grumous deposit on the walls and bottom of the tube, the upper portion of the liquid remaining clear, similarly to what is observed in the growth of some varieties of streptococci. There is a scanty growth on *potato* and *milk;* milk is not coagulated.

The bacillus of bubonic plague forms no gas in media containing sugar, and but little indol. It produces toxins, and the serum of animals immunized against the bacillus yields antitoxic substances.

Vitality.—The bacilli of bubonic plague withstand desiccation for from three to seven days; in water they die in from three to eight days according to its composition; in buried cadavera they retain their vitality for twenty-eight to thirty-eight days. Exposed to the action of direct sunlight they are destroyed in from three to four hours. They are killed by heating at 55° C. in ten minutes, and at 80° C. in five minutes. Corrosive sublimate (1 to 1,000) destroys the bacilli immediately.

Pathogenesis.—This bacillus is pathogenic for almost all animals, only pigeons being immune. Guinea-pigs, rats, and mice are the most susceptible animals; somewhat less so are monkeys, rabbits, cats, and horses; and still less so are dogs and cattle. Guinea-pigs when in-

EXPLANATION OF
PLATE XII

EXPLANATION OF PLATE XII.

FIG. 1.—Bacillus Anthracis from Cellular Tissue of Inoculated Mouse. Stained with gentian violet. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

FIG. 2.—Anthrax Spores from a Bouillon Culture. Double-stained preparation—with carbol-fuchsin and methylene blue. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

FIG. 3.—Bacillus of Tetanus from an Agar Culture. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

FIG. 4.—Bacillus of Glanders. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

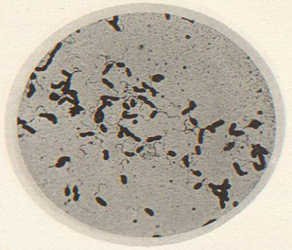
FIG. 5.—Spirillum of Asiatic Cholera (Comma Bacillus). From a culture upon starched linen at end of twenty-four hours, stained with fuchsin. × 1,000. Photomicrograph from Sternberg's "Bacteriology" by permission.

FIG. 6.—Spirillum of Finkler and Prior with Flagella. Agar culture. × 1,000. Photomicrograph from Bowhill's "Bacteriology" by permission.

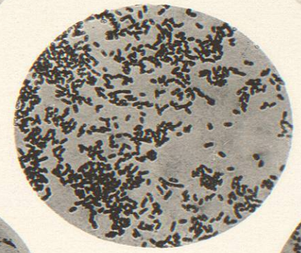
FIG. 7.—Bacillus of Bubonic Plague from Agar Culture, Showing Irregular Forms. × 1,000. Photomicrograph.

FIG. 8.—Bacillus of Bubonic Plague from Bouillon Culture, Showing Rods in Chains with Polar Staining. × 1,000. Photomicrograph.

I.
Bacillus Anthracis.

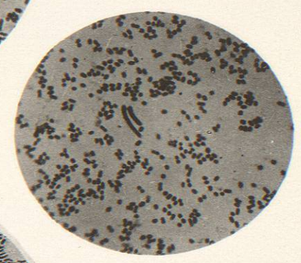


VI.
Spirillum Finkler-Prior.



IV.
Glanders Bacillus.

II.
Anthrax Bacillus,
with Spores.



VII.
Plague Bacillus.
(Agar Culture).

V.
Cholera Spirillum.

III.
Tetanus Bacillus.

VIII.
Plague Bacillus.
(Broth Culture)

Pathogenic Bacteria.