there is a special appearance of the culture that is shared by only a few other spirilla. At the upper portion of the gelatin there is a round cavity full of air, and as the surrounding gelatin has remained solid, the appearance is that of a bubble of air in the midst of the upper part of the gelatin and above the liquefied part. The appearance is wholly different when the organism found by Finkler is put under the same conditions, and equally so with the cheese spirillum of Deneke. The spirillum of Metschnikoff grows in very similar fashion in stab cultures in gelatin, and there have been isolated from water and other sources spirilla which cannot be distinguished from the cholera vibrio in this culture medium.

Morphology.—Slightly curved rods, with rounded ends, from 0.8 μ to 3 μ long, and about 0.3 μ to 0.5 μ broad. They are usually but slightly curved, like a comma, but are occasionally in the form of a half-circle, or two united rods curved in opposite directions, forming an **S**-shaped figure. Under certain circumstances these curved rods may develop into long spiral filaments, and in hanging-drop cultures the **S**-shaped figures may also be seen to form the commencement of a spiral. In stained preparations the spiral character of the long filaments is often obliterated, or nearly so. When development is very rapid, the short curved rods, or **S**-shaped spirals, only are seen, but in hanging-drop cultures, or in media in which the development is retarded by an unfavorable temperature, the presence of a little alcohol, and so on, the long spiral filaments are quite numerous, and it is quite generally agreed that the so-called comma bacillus is really only a fragment of a true spirillum.

By Löffler's method of staining, the rods may be seen to have from one to three terminal flagella. In old cultures the bacilli frequently lose their characteristic form, and become variously swollen and distorted. Hueppe has described the appearance in the course of the spiral filaments, of spherical bodies which he believes to be reproductive elements, the so-called arthrospores. These bodies are not functionally spores; for cultures containing them are no more resistant than those which contain spirilla without these bodies. The cholera bacillus stains with the usual aniline colors, but not so quickly as many other bacteria. It is best stained by a watery solution of fuchsin. It will not stain by Gram's method. Sections may be stained by Löffler's methylene blue.

Biological Characters.—It is an aërobic, facultative anaërobic, liquefying, motile spirillum, grows in the usual culture media at the room temperature, more rapidly in the incubator, does not develop above 42° C. or below 14° C., does not form endogenous spores. In gelatin plate cultures, at 22° C., at the end of twenty-four hours small white colonies may be perceived in the depth of the gelatin. These grow toward the surface, and cause liquefaction of the gelatin in the form of a funnel that gradually increases in depth, and at the bottom of which is seen the colony, in the form of a small white mass. As a result of this, the plates on the second or third day appear to be perforated with numerous small holes; later, the gelatin is entirely liquefied. Under a low power, the young colonies, before liquefaction has commenced, present a somewhat characteristic appearance. They are of a white, or pale yellow color, with a more or less irregular outline, the margins being rough and uneven; the texture is coarsely granular, and the surface looks as if it was covered with little fragments of broken glass, while the colony has a shining appearance. When liquefaction commences, an ill-defined halo is first seen to surround the granular colony, which by transmitted light has a peculiar roseate hue.

In gelatin needle cultures development occurs all along the line of inoculation, but liquefaction of the gelatin occurs at first only near the surface. On the second day, at 20° C., a short funnel is formed that has a comparatively narrow mouth, the upper portion containing air; just below this is the whitish viscid mass of the growth. At the end of from four to six days, the funnel, having increased in depth and diameter, may reach the walls of the test tube. In from eight to fourteen days the upper two-thirds of the gelatin is completely liquefied. Owing to the slight liquefaction occurring along the line of growth the first three or four days, the central mass that has formed along the line of inoculation settles down as a curved or irregularly bent yellowish-white thread, in the lower part of a slender tube filled with liquefied gelatin, the upper part of which widens out, and is continuous with the funnel above. (See Fig. 5 of Plate LIX.)

On the surface of nutrient agar a moist, shining, white layer is formed along the line of inoculation. Blood serum is slowly liquefied. On potato in the incubator, a rather thin semitransparent brown, or grayish-brown layer is developed.

In nutrient bouillon the development is abundant and rapid, especially in the incubator. The fluid is only slightly clouded, but the spirilla accumulate at the surface, forming a wrinkled membranous layer.

Sterilized milk is also a favorable culture medium. The milk, however, is not visibly altered by the growth of the bacilli.

In general this organism grows in any fluid containing a small quantity of organic material and having a slightly alkaline reaction. An acid reaction of the culture medium prevents its development as a rule, but it has the power of gradually accommodating itself to the presence of vegetable acids, and grows upon potatoes (in the incubator only) which have a slightly acid reaction. Abundant development occurs in bouillon which has been diluted with eight or ten parts of water, and the experiments of Wolffhügel and Riedel show that it also multiplies to some extent in sterilized river or well water, and that it preserves its vitality in such water for several months, but in milk or water containing other bacteria it dies out in a few days. This organism is destroyed, in recent cultures, in nutrient gelatin at 52° C., as determined by Sternberg, the time of exposure being four minutes. A few colonies only develop after exposure to a temperature of 50° C. for ten minutes. In Kitasato's experiments ten or even fifteen minutes' exposure to a temperature of 55° C. was not always successful in destroying the vitality of the spirilla, although in certain cultures exposure to 50° C. for fifteen minutes was successful. The low resisting power to heat, desiccation, and chemical agents, indicates that this organism does not form spores, and most bacteriologists agree that this is the case.

Hueppe has described a mode of spore formation which is different from that occurring among the bacilli, that is, the formation of so-called arthrospores. These are said to be developed in the course of the spiral threads, not as endogenous, refractive spores, but as spherical bodies with a somewhat greater diameter and somewhat more refractive; but this method of spore formation has not been observed by others who have investigated the question, and cannot be considered established.

The test for the presence of the cholera spirilla originated by Bujwid and by Dunham consists in the reddish-violet color produced in the bouillon cultures containing peptone, or in cultures in nutrient gelatin when a small quantity (five to ten per cent.) of sulphuric acid is added to the cultures. According to Fraenkel this test serves to distinguish it from the ordinary bacteria of the intestine, and from the Finkler-Prior spirillum, but not from Metschnikoff's spirillum. The reaction is shown by bouillon cultures which have been in the incubator for ten or twelve hours, and by gelatin cultures in which liquefaction has occurred. The sulphuric acid should be quite pure. The color quickly appears, and is reddish-violet or purplish-red. According to Salkowski, the red color is due to the well-known indol reaction, which in cultures of the cholera spirillum is exceptionally intense and rapid in its development.

The most satisfactory method for obtaining the "cholera-red" reaction—as we have had lately abundant opportunity to verify—is that of Beyerinck ("Cat. f. Bact. u. Parasit.," Bd. xii., S. 715) in which cultures are made in filtered neutralized one-half-per-cent. solution of commercial peptone, at 37° C. After twelve to twenty-

EXPLANATION OF
PLATE LIX.

EXPLANATION OF PLATE LIX.

FIG. 1.—Mucus from the Intestine of a Cholera Patient. Cover-glass preparation, stained with gentian violet. Magnified 800 diameters. (Fraenkel.)

FIG. 2.—Colony of Cholera Bacilli in Gelatin, after the Lapse of Seventy-two Hours. Magnified 75 diameters. (Plagge.)

FIG. 3.—Colony of Cholera Bacilli in Gelatin, after the Lapse of Seventy-two Hours. Magnified 170 diameters. (Koch.)

FIG. 4.—Cholera Bacilli from a Bouillon Culture at the Expiration of Twenty-four Hours. Cover-glass preparation stained with fuchsin. Magnified 1,000 diameters. (Koch.)

FIG. 5.—Needle Cultures of Cholera Bacilli in Nutrient Gelatin, at the expiration of one day, three days, four days, five days, seven days, and ten days, respectively. (From Koch and Gaffky: "Bericht über die Thätigkeit der zur Erforschung der Cholera im Jahre 1883 nach Egypten und Indien entsandten Commission," Berlin, 1887.)

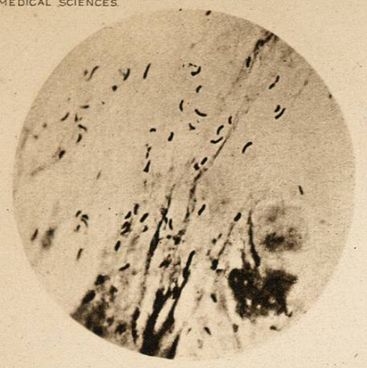

FIG.1.

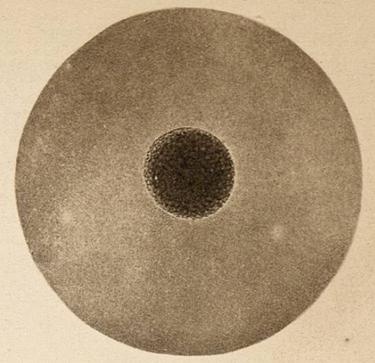

FIG.2.

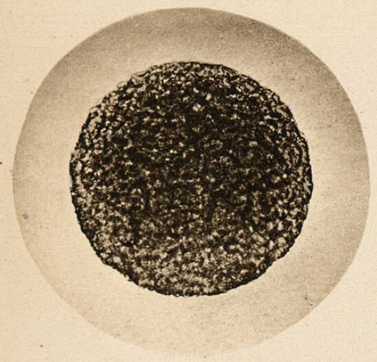

FIG.3.

FIG.4.

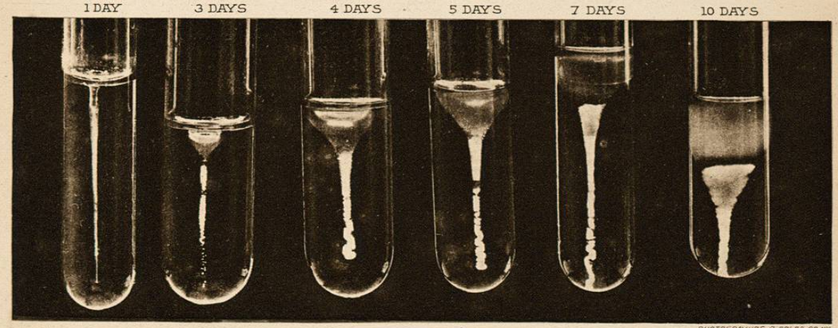

FIG.5.

ASIATIC CHOLERA.

CULTURES AND STAINED COVER-GLASS PREPARATIONS.
(AFTER KOCH AND GAFFKY.)

four hours the cultures are cooled, and from two to five drops of chemically pure sulphuric acid are added. If the spirillum be present, a very marked and beautiful rose-violet color is produced in the course of a few moments. In peptone-water cultures this indol reaction is perceptible as soon as the faintest clouding of the medium can be observed. Four to six hours' incubation at 37° C. may suffice.

A test which is said to distinguish cultures of the cholera spirillum from the spirillum of Deneke and that of Finkler-Prior has been suggested by Cahen. This consists in adding a solution of litmus to the bouillon, and in making the cultures at 37° C. The cholera cultures show on the following day a decoloration which does not occur at this temperature with the other spirilla named. For determining as promptly as possible whether certain suspected excreta contain the cholera spirilla, a little of the material may be used to inoculate greatly diluted bouillon, or a one-per-cent. peptone, one-half-per-cent. salt solution, gelatin plates being made at the same time. At the end of ten or twelve hours the cholera spirilla, if present, will already have formed a characteristic wrinkled film upon the surface of the bouillon. A little of this should be used to start a new culture in bouillon, and a series of gelatin plates made from it, after which the indol test may be applied. In the peptone solution mentioned above, the cholera spirilla seek the surface of the medium, and if cultivated at 37° C. a little of the material may be taken with a loop from the surface at the expiration of three hours and used for plate cultures or for examination in the hanging drop. The result of this, in connection with the morphology of the micro-organisms forming the film, and the character of growth in the gelatin plates, will establish the diagnosis, if the cholera spirillum is present in considerable numbers; if but few are present in the original material it may be necessary to make two or more series of plates and bouillon cultures before a pure culture can be obtained, and a positive diagnosis made. These tests will suffice for the purpose in times of epidemic cholera, but when there is doubt concerning the existence of cholera in the region, more rigorous identification is desirable. The virulence of the cultures may be tested on guinea-pigs and the agglutinative test with the serum of immunized animals applied.

Pfeiffer has shown that recent aërobic cultures of the cholera spirillum contain a specific toxic substance fatal to guinea-pigs in extremely small doses. This substance stands in close relation with the bacterial cells, and is perhaps an integral part of the same.

The spirilla may be killed by chloroform, by thymol, or by desiccation without apparent injury to the toxic character of this material. It is destroyed, however, by absolute alcohol, by concentrated solutions of neutral salts, and by the boiling temperature, and secondary products are formed which have a similar physiological action, but are from ten to twenty times less potent.

Similar toxic substances were obtained by Pfeiffer from cultures of the Finkler-Prior spirilla, and from the spirilla of Metschnikoff.

The spirillum is not found in the blood, nor in the various organs of individuals dead of cholera, but is always found in the discharges during life, and in the contents of the intestines examined immediately after death, frequently in almost a pure culture in the colorless rice-water discharges. They may persist in the discharges for twenty-three or more days, but usually disappear from the ninth to the twelfth day of the disease. Occasionally they may fail to appear in cultures for one or more days during the attack. It is evident that the morbid phenomena must be ascribed to the absorption of toxic substances formed during the growth of the spirillum in the intestine. In cases which terminated fatally after a very brief sickness Koch found but very slight changes in the mucous membrane of the intestine, which was slightly swollen and reddened; but in more protracted cases the follicles and Peyer's patches were reddened about their margins, and an invasion of the mucous membrane by the organisms was observed in properly stained sections. They penetrated especially the follicles of Lieberkühn, and in some cases were seen between the epithelium and the basement membrane. As a rule, the spirillum is not present in vomited material, but there are numerous exceptions to this rule on record. All observers have found the organism always present in cases of true cholera; on the other hand, very numerous control experiments fail to show its presence in the intestinal contents of healthy persons, when cholera is not prevalent, or in that of those dead from other diseases.

Nicati and Rietsch observed a certain degree of attenuation in the pathogenic power of the spirillum, after it had been cultivated for a considerable time at from 20° to 25° C., and the observation has since been made that cultures which have been kept up from Koch's original material have no longer the original pathogenic power. They also gradually change in biological characters upon prolonged cultivation, the colonies on gelatin plates being much less glistening and granular.

The organism most likely to be confounded with the cholera spirillum, and from which it must be differentiated by the methods of cultivation, as well as by the aid of the microscope, is the spirillum of Finkler-Prior, otherwise called the vibrio proteus. It was obtained by Finkler and Prior in 1884, from the fæces of patients with cholera nostras, after allowing the dejecta to stand for some days. Subsequent researches have not sustained the view that this spirillum is the specific cause of cholera nostras. Besides this spirillum many others, more or less resembling the spirillum of cholera, have been isolated. With the exception of the vibrio of Ivanoff (Migula), an organism incidentally isolated from the stools of a patient suffering from typhoid fever, none of these spirilla agglutinate with cholera immune serum. The colonies of this vibrio, however, do not closely resemble those of cholera on gelatin plates, being less granular and more filamentous.

Despite the numerous controversies on the subject since the first announcement by Koch that he considered his spirillum to be the cause of cholera, and the many assertions to the contrary, the relationship between the two is to-day admitted by practically all authorities. The opinion to this effect is based upon a number of reasons, some of which are as follows:

1. The spirillum of Koch has never been found in any other disease, a point that has been established by innumerable observations.

2. This spirillum is found in all cases of Asiatic cholera, without exception, provided that the case is not too far advanced. In such a case there has been a secondary infection, that has killed out the true organism and masked its effects. Ever since the first announcement of this discovery by Koch, observers have verified his assertions in all parts of the world where cholera has been investigated.

3. This spirillum is found in slight cases as well as in severe. It is present in the beginning of the attack, and it is located in the intestine, that is to say in the region especially attacked by the disease, and in which occur the initial and essential lesions.

The attempts to add to these arguments the more decisive one of successful inoculation experiments, were at the first not satisfactory in their results. Nicati and Rietsch were the first to secure successful results, which they obtained by introducing pure cultures of the organism into the duodenum of guinea-pigs, in which animals they had previously tied the ductus choledochus. By these experiments they obtained symptoms extremely analogous to those of true cholera, and Koch also succeeded in the same direction by passing the infectious material (pure cultures) into the stomach by an œsophageal catheter. In his experiments the animals had been previously narcotized by the injection of tincture of opium, to prevent peristalsis, and their stomachs had been made alkaline by the introduction of carbonate of soda, a solution to make the contents of the stomach