

tion does not consist in producing an increased power of resistance against the development of the infecting agent, but, similar to diphtheria, in bringing about an immunity to the effects of the tetanus toxin. The methods originally proposed by Behring and by Roux for producing a serum for the treatment of the disease consisted chiefly in weakening the tetanus toxin by means of chemical disinfectants (iodine trichloride, Gram's solution, etc.), so that when inoculated into the test animals they produced comparatively little reaction. At the present time pure unaltered toxin is injected either alone in small doses or along with antitoxin. After the first dose of toxin the animals acquire a certain tolerance which enables them to stand a dose of a less attenuated toxin or of a greater amount of unchanged toxin. Then by gradually increasing the doses or the strength of the toxin administered, the animals are finally able to bear injections of large quantities of the strongest toxin.

These immunizing experiments in tetanus have borne practical fruit, for it was through them that the principle of serum therapeutics first became known. It was thus shown that animals could be protected from tetanus infection by the previous or simultaneous injection of tetanus antitoxin, provided that such antitoxin was obtained from a thoroughly immunized animal; and from this it was assumed that the same result could be produced in natural tetanus in man. But unfortunately, the conditions in the natural disease are very much less favorable, inasmuch as treatment is usually commenced, not shortly after the infection has taken place, but often only on the appearance of tetanic symptoms, when the poison has already diffused itself through the body.

The tetanus antitoxin is prepared in the same manner as the diphtheria antitoxin—by inoculating the tetanus toxin in increasing doses into horses. The toxin is produced in bouillon cultures grown anaerobically. After ten or fifteen days the culture fluid is filtered through porcelain, and the germ-free filtrate is used for the inoculations. The horses receive 0.5 c.c. as the initial dose of a toxin of which 1 c.c. kills 250,000 gm. of guinea-pig, and along with this a sufficient amount of antitoxin to neutralize it. In five days this dose is doubled, and then every five to seven days, as rapidly as the horses can stand it, until they support 700–800 c.c. or more at a single dose. After some months of this treatment the blood of the horse contains the antitoxin in sufficient amount for therapeutic use. When the temperatures of the horses are normal and they have recovered from the dose of toxin last given, they are bled into sterile flasks and the serum collected.

Tetanus antitoxin is tested exactly as is diphtheria antitoxin, except that the standard unit is different. The test toxin used in the German method is one of which 1 gm. destroys 150,000,000 gm. of mouse. This is dissolved in 33½ c.c. of ten-per-cent. sodium chloride solution. Ten times the amount of antitoxic serum which neutralizes 1 c.c. of this dilution of the test toxin contains 1 unit of tetanus antitoxin. In the French method the amount of antitoxin which is required to protect a mouse from a dose of toxin sufficient to kill in four days is determined, and the strength of the antitoxin is stated by finding the amount of serum required to protect 1 gm. of animal. If 0.001 c.c. protected a 10 gm. mouse, the strength of that serum would be 1 to 10,000. Guinea-pigs are sometimes used instead of mice.

The dose of tetanus antitoxin for immunization is 10 c.c. of a serum of a strength of 1 to 1,000,000,000 unless the danger seem great, when the injection may be repeated after seven or eight days. For treatment it is well to begin with 50 c.c. and then, according to the severity of the case, give from 20 to 50 c.c. each day until the symptoms abate. The curative treatment in man has not been followed by very satisfactory results, owing to the fact already stated that the disease is generally too far advanced before treatment is commenced. From statistics collected by Lambert and others, however, of cases of tetanus treated with antitoxin, the remedy would seem to have been of undoubted practical use—so much so, at

least, that in all cases in which tetanus is suspected or in which dirt has been ground into serious contusions, in gunshot wounds, etc., preventive inoculations of the serum should be given. In certain parts of France where tetanus is very prevalent among horses, Nocard distributed tetanus antitoxin to sixty-three veterinary surgeons, who treated with it, for the prevention of the disease, 2,727 of these animals. Only one of this number became affected, and this horse was not inoculated until five days after being pricked in shoeing. Although the delay was too great to prevent the appearance of tetanus, yet the disease was of a very mild nature. During the same period 259 cases in animals that were not so treated were observed. These striking results would certainly seem to indicate that the remedy deserves a much more extensive consideration in the treatment of patients with immunizing doses of serum than has heretofore been given it—at least in neighborhoods where tetanus is not uncommon (fortunately it is a rare disease in man), and when the dirty condition of their wounds leads one to suspect the possibility of tetanus infection. The recently proposed method of injecting from 3 to 15 c.c. of tetanus antitoxin into the lateral ventricles has not so far shown itself to be superior to the intravenous or subcutaneous methods, and is not in general to be recommended. No bad results have followed the injection of the antitoxin when the serum was sterile and the operation was performed aseptically.

THE BACILLUS OF TYPHOID FEVER (*Bacillus Typhi Abdominalis*).—This organism was first observed by Eberth, and independently by Koch, in 1880, in the internal organs of typhoid cadavera. It was obtained in pure culture by Gaffky in 1884; and has also been found during life in the blood, urine, and feces of typhoid patients. Its etiological relationship to typhoid fever has been somewhat difficult of demonstration from the fact that, although pathogenic for many animals when artificially inoculated, it has not been easy to produce infection or give rise to lesions corresponding to those occurring in man. Still the results which have been obtained under certain conditions, together with the specific reactions of the blood serum of typhoid patients, and the constant presence of the bacillus in the spleen, blood, and excretions of the sick during life, have finally established, on a scientific basis, that this organism is the chief cause of typhoid fever.

**Microscopical Appearances.**—As met with in the organs of man and animals the typhoid bacilli are short, plump rods with rounded ends. They vary in size, being from 1 to 3  $\mu$  long and 0.5 to 0.8  $\mu$  broad, usually occurring singly, but sometimes growing into long threads, especially in certain culture media, as in potato. They are generally longer and somewhat more slender than the bacillus coli under similar conditions. (See Plate X., Fig. 7.)

**Motility.**—Actively motile, especially the short bacilli, each rod possessing from eight to fourteen flagella attached to the sides and extremities of the cells. The longer threads have a sinuous and more sluggish motion. (See Plate X., Fig. 8.)

**Spore Formation.**—Does not form spores. In stained preparations, particularly when grown on potato, refractive granules may be seen at the ends of the rods, which stain more intensely, and in the body of the cells "vacuoles" which remain unstained. These so-called Gaffky's spores, however, are not true spores, as the bacilli containing them show even less resisting power than the homogeneous bacilli found in other cultures, but are probably involution forms.

**Staining Reactions.**—The typhoid bacilli stain with the ordinary aniline colors, but a little less readily than do most other organisms, though this is not constant. They are decolorized by Gram's solution.

**Biological Characters.**—The bacillus typhosus grows most luxuriantly in the presence of oxygen, but oxygen is not essential to its development (facultative anaerobic), it grows fairly well also in an atmosphere of CO<sub>2</sub>. Its growth on the ordinary culture media is similar to that of the bacillus coli communis, but somewhat slower and

not quite so abundant; in contradistinction to most other pathogenic micro-organisms, it grows well on slightly acid media. Below 10° C. it does not develop, its optimum temperature being at 37° C.; over 40° and below 30° C. its growth is retarded.

**Growth in Gelatin.**—In gelatin plates the deep colonies are not characteristic; they are small, punctiform, and sharply circumscribed, of a yellowish-brown color and finely granular in structure. The superficial colonies, however, particularly when young, are quite characteristic; they form a bluish-white, transparent, iridescent coating on the medium, with irregular outline, denser in the centre than at the periphery, and exhibiting under a low power a brownish color and wrinkled appearance. The gelatin is not liquefied.

In gelatin stab cultures the growth is mostly confined to the surface; it is thin, thready, often slightly granular, extending along the track of the needle and gradually reaching out to the sides of the tube; white to yellowish brown in color, iridescent and transparent. There is no liquefaction.

**Growth in Agar and Blood Serum.**—Not distinctive. **Growth in Bouillon.**—This medium is uniformly clouded, but the clouding is not so dense as by the colon bacillus. After eighteen to twenty-four hours' growth a sediment is frequently developed, and a film forms on the surface, with a slightly acid reaction.

**Growth in Potato.**—The growth in this medium is generally considered to be very characteristic, but it varies considerably. The typical growth is a slightly moist, almost invisible, but luxuriant layer, usually covering the surface of the potato, and when scraped with the needle is tough and tenacious. Sometimes, however, the development is restricted, not very luxuriant, and of the same color as the medium. Again, it may be quite heavy, of a yellowish-brown color with a greenish halo, and similar to that of the colon bacillus. These variations in growth are thought to be due to the reaction (alkalinity) of the potato.

**Milk** is not coagulated, but some acid is produced by the typhoid bacillus. The bacillus coli communis, on the contrary, causes coagulation of milk in twenty-four to forty-eight hours at 37° C.

**Vitality.**—The typhoid bacilli withstand desiccation for months; according to Uffelmann in dried earth, clothes, etc., for two months or more. In dust, however, they do not seem to live so long. They resist cold remarkably well; freezing and thawing repeatedly under favorable conditions finally kills them. They are destroyed by heating to 60° C. in ten minutes and at higher temperatures still more rapidly. In feces the bacilli retain their vitality for weeks or months, depending upon the number of putrefactive organisms present. In oysters they have remained alive for a month. In water which has been sterilized they live for many days; in ordinary water they are destroyed, by the concurrence of other bacteria, in about fourteen days; in running water this destruction takes place more rapidly. It thus appears that, under favorable circumstances, protected from light and other deleterious influences, the typhoid bacilli may retain their vitality outside of the body for a considerable length of time. But they may live also in the human body for a long time; Sahli has found them in the pleural exudate fifty days from the beginning of the disease, and Heintze observed them in a case of typhoid fever in peritestic pus ten months after convalescence.

**Chemical Effects.**—The typhoid bacillus produces no pigment or odorous substances. It reduces litmus solutions; converts nitrates into nitrites, the latter being gradually decomposed; forms lactic acid from grape sugar, but does not produce gas from carbohydrates; produces H<sub>2</sub>S abundantly, but does not produce indol. The cultures are rich in toxins which, when freed from germs by filtration, are active disease producers.

**Pathogenesis.**—Although the typhoid bacillus is pathogenic for mice, guinea-pigs, rabbits, goats, etc., which when inoculated with virulent cultures die; showing

symptoms of spasm, falling temperature, and diarrhoea, no experiments so far have produced in animals the typical lesions of typhoid fever in man. Certain experiments have indicated that the presence of other bacteria in the body, and of exposure to the action of poisonous gases in lowering the natural resistance of the individual, may render him more susceptible to typhoid infection. But whatever conclusions may be drawn from these results with regard to the typhoid process in animals, in the human subject typhoid fever is now generally recognized as a true infection, caused by the invasion and growth of typhoid bacilli in the body. This disease belongs to the class of infections known as *metastatic*—that is to say, diseases in which the specific infective organisms do not abound in the circulation, as in septicæmia, nor remain localized in one situation, but are distributed through the body in groups, the characteristic lesions of typhoid fever being in the lymphatic structures of the intestines, viz., the solitary follicles and patches of Peyer, the mesenteric glands, and the spleen; the liver and kidneys are less commonly affected.

Outside of the body the typhoid bacilli have been found so far only in comparatively few instances in water and soil, which have become contaminated with typhoid dejections; also recently in milk. They have never been found in healthy persons, except when convalescent from typhoid fever. In typhoid patients they have frequently been detected in the spleen and other organs (kidneys, liver, gall duct, etc.), the blood, urine, and feces. They are most easily isolated from the spleen and lymphatic glands; they are often difficult to isolate from the excretions. The typhoid bacillus may give rise to the most varied complications along with the clinical symptoms of typical typhoid fever; it has been demonstrated to be the cause of suppurative inflammations of the spinal cord, of the brain and its membranes, of the lungs and kidneys, and of different suppurative processes, erysipelas, abscess, etc., in typhoid patients. The pyogenic functions of the typhoid bacillus are indeed no longer disputed. But at the same time in many cases of mixed infection in typhoid fever, the other pus cocci (streptococcus, staphylococcus, pneumococcus, etc.) are no doubt concerned in the production of the complications of the disease.

With regard to the *mode of infection* by the typhoid bacillus, there is no doubt that it is principally by way of the mouth and stomach to the intestines through drinking water, etc. In a case reported by Mayer in which death occurred on the second day of the disease, there were found on autopsy lesions of the lungs, spleen, kidneys, and intestines and great enlargements of the solitary follicles and patches of Peyer, but nowhere a trace of necrosis or loss of substance nor enlargements of the mesenteric glands. Microscopically an extraordinary deposit of characteristic typhoid bacilli was observed in the submucosa and interstitial spaces of the muscular tissue. In other cases, however, no intestinal lesions have been present, only a localization of bacilli and changes in the mesenteric glands and spleen revealing the nature of the infection. Here absorption probably took place more rapidly than usual, the bacilli not multiplying to any extent in the intestines. But not only do those cases which have been examined bacteriologically and pathologically, but also the epidemiological history of typhoid fever, prove beyond question that the chief mode of invasion of the specific bacillus is by way of the mouth. The infective material being discharged in the feces and urine of typhoid-fever patients—in the latter of which especially the bacilli often persist for weeks or months—contaminate the water supply, articles of food, hands of nurses and attendants, etc., and thus spread infection from place to place. On this account the disinfection of the dejections of typhoid patients and convalescents cannot be too carefully looked after.

**Immunization.**—Specific immunization against experimental typhoid infection has been produced in animals by the usual method of injecting at first small quantities of the living or dead typhoid culture and gradually in-

creasing the dose. The blood serum of animals thus immunized has been found to acquire protective and curative bactericidal and possibly feeble antitoxic properties against the typhoid bacillus. These characters have also been observed in the blood serum of persons who have recovered from typhoid fever; and recently the attempt has been made to employ the typhoid serum of immunized animals or dead cultures for the cure and prevention of typhoid fever in man, but no marked results have been obtained.

**Specific Reactions.**—The following specific reactions have been utilized for the differential diagnosis of the typhoid bacillus from other similar organisms, and as an aid to the clinical diagnosis of obscure cases of typhoid fever:

1. The typhoid bacillus does not produce indol.
2. It does not produce fermentation or gas from media containing grape sugar, milk, or cane sugar.
3. On lactose litmus agar it grows usually as pale blue colonies, but occasionally causes slight redness of the surrounding medium.
4. **Widal's Serum Reaction.**—This reaction is based upon the fact, first observed by Pfeiffer, Gruber, and Durham, but since practically applied on a more extended scale by Widal, that living and actively motile typhoid bacilli if placed in the diluted blood or serum of a patient suffering from typhoid fever, within a very short time lose their motility and become aggregated into clumps. Either dried blood or serum may be used for the demonstration of the reaction. The blood is obtained by pricking with a needle the skin (previously disinfected) covering the tip of the finger or ear, and allowing two drops to fall on a glass slide, one near either end, where they dry. Fluid blood serum may be obtained in two ways: First, the tip of the finger or ear is pricked and the blood as it issues is allowed to fill by gravity a capillary tube having a central bulb, the ends of the tube being then sealed by heat and the serum allowed to separate from the clot. Second, a small piece of cantharides plaster is applied to the skin at some spot on the chest or abdomen, and from the blister thus formed in six to eighteen hours, the serum is collected in a capillary tube, the ends of which are then sealed. The latter method is the best, for the serum obtained is clear, free from blood cells and fibrin, which somewhat obscure the field on examination in the hanging drop, and is admirably suited to the test. Dried blood, however, obtained as above described answers all practical purposes of diagnosis.

The method of performing the serum test is as follows: A dilution of the blood or serum is first made in the proportion of one to ten. In the case of dried blood, it is dissolved in a little water and then mixed with the typhoid culture (eighteen to twenty-four hours old), the degree of dilution being guessed by the color. By previously making test solutions of dried blood in water of known proportions and noting the color the dilution may be approximately gauged. If serum is used which is preferable, not only because there is less fibrinous deposit but also because it is possible to make the dilution more accurately, one part of serum is added to nine parts of the broth culture. This should contain living and actively motile isolated bacilli. If there is no reaction when the mixture is observed in the hanging drop—that is to say, if within five minutes no marked change is noted in the motility of the bacilli and no considerable clumping occurs—the result may be regarded as negative, and no further test of the specimen is necessary. If complete clumping and immobilization of the bacilli occur within five minutes, this is a marked immediate typhoid reaction, and though this test is ordinarily sufficient for a positive diagnosis, the reaction may be confirmed with higher dilutions up to one to twenty, or more, if desired. If, however, upon examination of the mixture there is no marked reaction, but the bacilli only show in the first few minutes an inhibition in their motility and a tendency to clump, not complete within five minutes, it becomes necessary to test this with dilutions up to one to twenty, in order to measure the strength of the reaction.

If in the one-to-twenty dilution a complete, distinct reaction takes place within thirty minutes, the result may also be considered positive, that is, that the blood or serum has come from a case of typhoid infection, while if a less marked reaction occurs it should be regarded as only probably typhoid, and another specimen should be requested. The time allowed by many observers for the development of the reaction with the higher dilutions is from one to two hours, but thirty minutes, in our opinion, is a safer and sufficient time limit. Positive results obtained in this way may be accepted as conclusive evidence of the recent or previous existence of typhoid infection in the patient. A former attack of typhoid fever within a period of several months or one or more years exceptionally vitiates the value of the reaction. On the other hand, the absence of reaction in any one examination does not exclude typhoid; so that, if the case remains clinically doubtful, repeated examinations should be made. The Widal reaction, though not infallible, when performed with due regard to the avoidance of every possible source of error, is as reliable as any other bacteriological test at present in use, and is of inestimable value as an aid to the clinical diagnosis of irregular or mild cases of typhoid infection. It is simple and easy of performance by any one versed in bacteriological technique. The serum reaction is never present in other diseases or in healthy persons, if correctly made and in the proper dilution, as is so often the case with Ehrlich's diazo reaction. It is better adapted for general employment than are any of the cultural methods now in use for isolating the bacillus from the feces or urine. It is certainly safer than spleen puncture, and it is not so difficult as, though far more reliable than, the leucocyte count. The reaction does not appear, as a rule, during the first few days of the disease, but it is usually manifest before the rose-colored eruption appears, though occasionally it is very late in appearance (that is, not till the fourth or fifth week and sometimes only during a relapse), and in rare cases may be entirely absent. Although a negative result, therefore, has but little significance, a positive reaction when present—previous typhoid being excluded—is almost as strong evidence of the existence of the specific infection as the actual demonstration of the typhoid bacilli.

**THE COLON BACILLUS (*Bacillus Coli Communis*).**—This organism was first described by Emmerich (1885), who obtained it from the blood, organs, and intestinal discharges of cholera patients at Naples under the name *Bacillus Neapolitanus*. It has since been found to be a normal inhabitant of the intestinal canal of man and many animals. A number of similar bacterial species are now often spoken of as the *coli* group of organisms.

**Microscopical Appearances.**—The size and shape of the colon bacillus vary considerably according to the culture media (age, composition, etc.) from which it is derived. The typical form is that of short rods with rounded ends (0.4 to 0.7  $\mu$  in diameter and 1 to 3  $\mu$  in length); but sometimes the rods are so short as to be almost spherical, and again oval or thread-like forms may occur. The bacilli are found singly, joined together in pairs, rarely associated in short chains. In unfavorable culture media in stained preparations polar granules and vacuoles are frequently present, supposed to be due to degenerative changes in the protoplasm. (See Plate XI, Fig. 1.)

**Motility.**—The rods possess numerous long flagella, but usually very sluggish movements.

**Spore Formation.**—Absent.

**Staining Reactions.**—Stains readily with the ordinary aniline colors; is quickly decolorized by Gram's solution.

**Biological Characters.**—This organism grows best in the presence of oxygen, but also, though less luxuriantly, without oxygen and in an atmosphere of CO<sub>2</sub> (facultative anaerobic). It develops rapidly on almost all culture media (best in media containing sugar) even at room temperature; optimum temperature 37° C. It grows fairly well in slightly acid media, but itself produces so much acid that it is sometimes destroyed in this way. It is almost impossible to distinguish culturally the bacillus

coli from the bacillus typhi, except that the growth of the former is somewhat more abundant under similar conditions.

**Growth in Gelatin.**—In *gelatin plates* colonies are developed in from twenty-four to forty-eight hours, which resemble greatly the colonies of the typhoid bacillus, except that they are larger for the same period of growth. The deep colonies are round, oval or "whetstone" shaped, finely granular, almost homogeneous in structure, and of a pale yellowish to brownish color, at first; later they become denser, darker, and more coarsely granular. The surface colonies appear as small, dry, irregular, flat, iridescent points with wavy bent borders. In *stab cultures* the growth usually takes the form of a nail with flattened head, the surface extension soon reaching out to the sides of the tube. The gelatin is not liquefied.

**On agar and blood serum** an abundant, soft, grayish-white layer is quickly developed in the incubator, but the growth is not characteristic.

**In bouillon** the colon bacillus produces diffuse clouding with sedimentation; a pellicle is sometimes formed on the surface; a decided fecal odor is often noticed in old cultures.

**Milk** is usually coagulated with the production of gas and acid.

**On potato** the growth is rapid and abundant, appearing after twenty-four to thirty-six hours in the incubator as a yellowish-brown to dark cream-colored deposit on the surface. The growth may, however, be scanty or absent at times.

**Chemical Effects.**—This bacillus forms pigment only on potato. Ill-smelling substances are developed on agar, gelatin, occasionally in old bouillon cultures, but particularly on potato media. The bacillus coli grows rapidly in media containing carbohydrates (grape and milk sugar, etc.), causing active fermentation with liberation of gas (CO<sub>2</sub> and H<sub>2</sub>). Cultivated in solid media, to which glucose has been added, the gas production is recognized by the appearance of numerous bubbles; in fluid culture media it may be demonstrated in the fermentation tube. Grown on lactose-litmus agar, the colonies are pink and the surrounding medium is changed from blue to red, showing the production of acid. The colon bacillus produces in bouillon and peptone solutions H<sub>2</sub>S and indol. It converts nitrates into nitrites. Urea is decomposed by many species of this group.

**Vitality.**—Similar to that of the typhoid bacillus, but is more resistant to the action of acids, formalin, and other chemical disinfectants. Thermal death point 60° C. in ten minutes' exposure.

**Pathogenesis.**—The colon bacillus is pathogenic for mice, guinea-pigs, and rabbits in varying degrees according to the strength of the virus and mode of inoculation; the results of animal inoculations, however, as with the typhoid bacillus, cannot always be predicted with certainty. The more rapidly death ensues the greater is the number of bacilli found in the body; they are always more abundant in the abdominal cavity than in the blood,—in other words, the result is due to the toxic rather than to the infective properties of the culture used. The lesions produced are those of enteritis; the duodenum and jejunum are found to contain fluid, the spleen is somewhat enlarged, and there is marked hyperemia and ecchymosis of the small intestines, together with swelling of Peyer's patches.

**Immunization** against colon infection is easily produced in the usual way by the inoculation of gradually increasing doses of cultures of living or dead bacilli.

The bacillus coli communis is a common inhabitant of the intestines of man and animals, being found in the feces, milk, bile, etc. Of thirty-two cadavers of healthy individuals examined twenty-four to thirty-six hours after death it was found in sixteen, especially in the liver and kidneys. It is also frequently met with in river water and food, so that it is one of the most widespread saprophytic bacteria. Formerly it was thought that the presence of the bacillus coli in water was sufficient proof of its contamination by feces, and thus of its possible

contamination also by typhoid bacilli. But recent investigations have shown that there are no grounds for this assumption, as the colon bacillus may reach the water from many different sources. At the same time, in a general way, drinking water found to contain colon bacilli may be regarded as unfit for human consumption.

This organism is associated with many diseases especially of the abdominal organs, though it is not positively known what etiological relation, if any, it bears to these affections. It has been found in peritonitis, appendicitis, cystitis (partly alone, particularly when the urine is acid, and partly together with the proteus vulgaris and other bacteria), urethritis, pyelonephritis, etc. The colon bacillus has been assumed to be the cause of *cholera nostras* and *cholera infantum*, but the investigations of Booker, Baginsky, Escherich, and Flügge would seem to indicate that these diseases are of a much more complicated origin, being due probably to certain ferments and toxins in the intestines produced, not by any specific micro-organisms, but by the ordinary putrefactive bacteria, among which the B. coli and B. proteus vulgaris are the most commonly present. The cause of infections of the gall ducts and multiple abscesses of the liver is also explained in this way. Puerperal fever is not infrequently due, in part at least, to infection of the vagina or uterus by the colon bacillus. Other diseases to which this organism seems to stand occasionally in relation are endocarditis, meningitis, tropical abscess of the liver, bronchopneumonia, fetid bronchitis, amygdalitis, etc. In these diseases the bacillus coli communis has been found sometimes alone, but usually associated with other pathogenic bacteria in such numbers that it must be considered a factor in the etiology of the affections, and in some cases there is reason for belief that it may be the primary cause. Though further study is required to show the specific pathogenic properties of this micro-organism, it is evident that under certain conditions it may become pathogenic to man.

**Differential diagnosis** between the B. coli and B. typhi abdominalis: The following characteristics and tests constitute the chief means of differentiating these two similar micro-organisms, though none of them alone can be depended on:

1. The motility of the colon bacillus is, as a rule, not very pronounced, sometimes absent; that of the typhoid bacillus is usually very active.
2. On gelatin plates the colon bacillus develops more rapidly and luxuriantly than the typhoid bacillus, and on potato it grows more abundantly, being almost always visible.
3. The colon bacillus coagulates milk with acid reaction within twenty-four to forty-eight hours; the typhoid bacillus does not coagulate milk.
4. The colon bacillus causes fermentation with production of gas in media containing sugar; the typhoid bacillus does not.
5. In nutrient agar or gelatin containing lactose and litmus tincture and of a slightly alkaline reaction, the color of the colonies of colon bacillus is pink and the surrounding medium red; while the colonies of typhoid bacillus are blue and there is little or no reddening of the medium.
6. The colon bacillus produces indol in cultures of bouillon or peptone; the typhoid bacillus does not.
7. When a twenty-four-hour-old bouillon culture of the colon bacillus is mixed with the blood or serum of a patient suffering from genuine typhoid fever, in a dilution of one to ten or more, after the first week of the disease, the Widal reaction is negative; cultures of the typhoid bacillus treated in the same manner and examined in the hanging drop give the characteristic agglutination and clumping of the bacilli.
8. Finally, we have the special media, devised respectively by Hiss, Capaldi, and Elsner, for isolating the colon and typhoid bacilli, in which we may observe their differences of growth in plate and tube cultures. These will be referred to more in detail elsewhere.