Original Articles.

THE DIFFERENTIATION OF THE BACILLUS OF TYPHOID FEVER.¹

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For several years it was believed that the most marked and sufficiently diagnostic characteristic of the typhoid fever bacillus was its growth on sterilized potato. In two days at blood temperature, or in three to four days at room temperature, the inoculated potato gives a luxuriant, but to the unaided eye, completely invisible growth. The entire surface assumes a moist, shining appearance but is not otherwise changed. If a small portion of the surface is removed with a sterilized platinum needle, and examined in a drop of water under the microscope, a large number of bacilli moving about very energetically are to be seen. It was thought for some time that this was a means of differentiation from all other species of bacteria. The results of many months' work in this laboratory, however, confirm the conclusion of several investigators abroad which have indicated that this is not the case, and for two reasons: first, because the typhoid bacillus does not invariably give this invisible growth, and, second, because there are several species very similar to the typhoid bacillus in their growth on the usual culture media, which under some circumstances present the same appearance when grown on sterilized potato. Of these so-called "pseudo-typhoid" bacilli there are at least five species in the water of the Merrimack River at Lawrence. Like other observers, I have found that this characteristic of the typhoid bacillus is obtained only when the potato is slightly acid; slightly alkaline potato giving a gray or yellowish growth along the inoculation line. Moreover, as different specimens of potato appear to vary in their acidity or alkalinity, and as the same variety changes in this respect during the different seasons of the year, the potato test is not only to be no longer considered diagnostic — it is often contradictory.

At the Lawrence Experiment Station of the State Board of Health of Massachusetts, under the direction of Mr. Hiram F. Mills, many experiments have been made upon the life-history of the typhoid bacillus in milk and in water, and upon the possibility of its passage through sand-filters. Realizing the uncertainty of the differentiation of this micro-organism by means of the potato test, a comparative study of the different species of bacteria in the water of the Merrimack River at Lawrence, of which there are more than thirty, was systematically undertaken side by side with cultures of the typhoid bacillus. To this end it became necessary to make a thorough investigation of the typhoid bacillus itself. The numerous special methods and media proposed for its differentiation were studied and their diagnostic value ascertained. The observations of its characteristics and its behaviour upon the various culture media now employed in this laboratory are as follows:

Morphology.—When grown on agar in tubes for one week at 20° C. it appears to be a plump bacillus, with rounded ends, about 1 μ long and 0.6–0.8 μ in diameter. When grown in bouillon at blood temperature for two days it is a bacillus 1.5–2.5 μ long and 0.5–0.6 μ in di-

ameter. Spore formation and involution-forms under the above conditions, have never been observed. Hanging drop preparations from bouillon cultures, at blood temperature, show that the bacilli possess very lively movements both of rotation and translation. The motility of the bacilli, when taken from agar cultures grown at blood temperature, is very constant but somewhat less marked, but from agar cultures grown at 20° C., it is not only less marked but it is not uniformly present.

While the motility of the typhoid bacillus is one of its most marked characteristics and is an essential number in the series of characteristics which enables the bacteriologist to identify this micro-organism, yet in water, sewage and other sources are found species of bacteria which possess this characteristic to a very similar degree. Moreover, it has been learned from the repeated determinations of the species of bacteria in numerous samples of water that an element of variability in the morphological appearances of bacteria arises when cultures are grown on media of different composition, of different degrees of alkalinity, for different periods of time, at different temperatures. Accordingly, in order to make microscopical observations of value, a standard method of procedure should be adopted, making all conditions as nearly parallel as possible.

Relation to Temperature.—The typhoid bacillus can be grown in culture media at temperatures varying from 10° C. to 45.5° C. Its optimum temperature lies between 37° C. and 39° C. It has been found that at the extreme limits of temperature the bacillus grows better in liquid than in solid culture media.

Description of Growth on the Different Culture Media.—On the gelatine plate the bacillus grows and forms colonies in the usual time, namely, forty-eight to seventy-two hours, at 20° C. The colonies lying deep in the gelatine are small, white, spherical or spindle-shaped and sharply outlined. Those on the surface are larger, bluish-white, with slightly irregular outline; under the microscope the colonies often appear to have ridges or folds. In some cases a centre is visible but it is the exception. The gelatine is never liquefied. It was thought at one time that the typhoid colony was very characteristic with its yellowish centre, and bluish veil of irregular outline with ridges like the veining of a leaf. It is now known that the colony does not always present this characteristic appearance and, further, that there are several species of bacteria in water which cannot be distinguished from the typhoid bacillus by their growth on the gelatine plate.

When grown in a gelatine tube a moderately conspicuous, gray growth appears along the inoculation line, while at the surface there is a thin, gray skin of irregular outline which spreads toward the wall of the tube.

On the agar plate incubated at 38° C. the surface colonies are slightly irregular, bluish-white, sometimes having a white centre. Those below the surface appear yellowish-white and are usually oval. On the inclined agar tube the typhoid bacillus gives a well-defined, smooth, moist, grayish growth.

When grown in sterilized milk the typhoid bacillus produces no coagulation and but a slight amount of acid. It is not possible to detect the acid with litmus but with phenolphthalein which is a more delicate indicator for organic acids than litmus, it can be shown that a slight acid fermentation takes place. The milk

¹ From a forthcoming Report of the State Board of Health.

test is manipulated as follows: The milk, drawn fresh from the cow and as nearly sterile as possible, is taken at once to the laboratory, tubed and sterilized—five cubic centimetres being carefully measured into each test-tube. The tubes are sterilized for three-quarters of an hour on three successive days. From time to time the apparatus and doubtless the atmosphere of the laboratory contain spore-forming bacteria which are killed with great difficulty at steam temperature. Notwithstanding the precautions mentioned above, I have frequently found that many of the tubes of milk were not sterile. It was also observed that the species which were not killed at steam temperature produced coagulation. Accordingly after the tubes have been sterilized three times they are placed in a thermostat at 38° C. for twenty-four hours. After another sterilization the tubes which had a growth in them are found to be coagulated and are discarded. Although many weeks may go by without having difficulty in this direction, the importance of having sterile milk, and of knowing that it is such, well repays the additional labor.

The tubes are inoculated and the cultures allowed to grow for two days at 37° C., or for four days at 20° C. At the end of this time they are examined for coagulation, after which they are placed in boiling water for five minutes and the coagulation, if any, again noted. The second observation gives uniformly constant results for the same species, whereas, before boiling, the coagulation by many species is sometimes positive and sometimes negative even after they have grown in the milk for a much longer time. The great advantage of this procedure is well illustrated in the case of the *bacillus coli communis* which ordinarily takes from two to seven days to coagulate milk. If, however, a culture is allowed to grow for twelve hours at 38° C., and is then placed in boiling water coagulation at once follows. In explanation of this fact it is to be remembered that the caseine of milk is precipitated by acid (and re-dissolved by alkali) and that it is coagulated by heat in the presence of acid; therefore, in some cases, probably, the acid although unable to precipitate the caseine is sufficient to coagulate it in the presence of heat. The fact, however, that in some cases a small amount of acid effects precipitation before heating, while in other cases a comparatively large amount of acid produces no coagulation after prolonged heating, points to further chemical changes.

After noting the coagulation, the milk is placed in an evaporating dish and the amount of acid determined by titration against a twentieth-normal solution of caustic potash using phenolphthalein as an indicator. As is well-known, normal milk is acid with this indicator, the five cubic centimetres of milk requiring about two cubic centimetres of a twentieth-normal solution of caustic potash for neutralization. This blank, which is determined for each lot of milk, is of course deducted from the amount of caustic potash required to neutralize each tube of milk after the bacteria have grown in it. It will be readily seen that there is a great advantage in using this indicator, inasmuch as the blank is sufficiently great to enable alkali, if present to a moderate extent, to be estimated as well as if the fermentation had been an acid one.

When grown in a peptone solution containing potassium nitrate, the typhoid bacillus reduces the nitrate to nitrite. The explanation of this fact seems to be that, among the decomposition products of the

peptone, some reducing agent or agents are formed—the nitrate being used simply as an indicator. It is not clearly known what the reducing agents are, but the test is of great value to the bacteriologist as the works of Heraeus, Frankland and Jordan have indicated. After many hundreds of trials upon the typhoid bacillus the test has never been known to fail, if made with proper manipulation. This method is also of great service in the differentiation of other species, as from the single test three possibilities may result:

(1) The nitrate may remain as such. (2) It may be reduced to nitrite. (3) It may be reduced completely; that is, to ammonia or to free nitrogen.

The solution is prepared by dissolving one gramme of peptone and two-tenths of a gramme of potassium nitrate in one litre of city water. The reason that this water is used rather than distilled water is because it contains the phosphates and other inorganic salts which appear to be necessary for the growth of the bacteria. The solution is rendered alkaline to the same degree as ordinary nutrient gelatine, tubed, sterilized, inoculated and the bacteria are allowed to grow for one week at 20° C. To determine in what condition the nitrogen is present, after the growth of the bacteria, a test for nitrites is first made. A cubic centimetre of the solution is removed to a clean test-tube, containing about ten cubic centimetres of distilled water free from nitrites. One cubic centimetre each of strongly acid, sulphonilic acid and of naphthylamine hydrochlorate solution are added; and nitrites, if present, are indicated by a pink color. If nitrites are present, then the determination is sufficient; but, if they are absent, a determination of nitrates must be made in order to learn, by their absence or presence, whether there has been a complete reduction, or none at all. To make the nitrate determination, the tube containing the solution is inverted and the small amount of solution remaining is evaporated to dryness. Phenolsulphonic acid, water and caustic soda are successively added and the presence of nitrates is indicated by the production of a yellow color.

For some time it has been known that the presence of glucose enables facultative anaerobes to grow luxuriantly in the absence of oxygen. Taking advantage of this fact, Dr. Theobald Smith,² of Washington, has devised a method which we have found to be of the greatest value for the diagnosis of the typhoid bacillus. The solution used contains two per cent. glucose, one per cent. peptone, five-tenths per cent. common salt, and is rendered alkaline to the same degree as the nutrient gelatine used in this laboratory. In place of the U tubes, such as are used in the determination of sugar in urine, recommended by Smith, small test-tubes bent have been found to answer the purpose very nicely. The tubes are bent at about one inch from the closed end, forming an interior angle of 75°. The solution is poured into the tube till the closed arm is filled and there stands about a half-inch in the open one. The tube is plugged, placed in a wire basket so that the closed arm will be in an inclined position, and sterilized in the usual manner. After boiling, the air in the closed arm rises to the upper end and must be removed at once to prevent reabsorption. The tubes are again placed in a basket, after inoculation, so that the closed arm shall be in an inclined position.

There are three ways, as Smith has shown, in which bacteria may grow in this tube: (1) obligate aerobes,

² *Centralblatt für Bakteriologie*, Bd. vii, p. 502; *Ibid.*, Bd. xi, p. 367.

and facultative anaërobes which are not motile, do not grow at all in the closed arm. (2) Facultative anaërobes which are motile grow into the closed arm and produce a turbidity. The typhoid bacillus is an example of this class. (3) Some facultative anaërobes, which are motile, grow into the closed arm producing a turbidity as do the last class, but they also liberate gas. *Bacillus coli communis* is an example.

This test has been found to be of special value in this laboratory as there are very few non-liquefying bacteria present in the water of the Merrimack River which belong to the same class as the typhoid bacillus.

The observations obtained from the above tests, taken altogether, have proved sufficient to differentiate the typhoid bacillus beyond all reasonable doubt from every species of bacteria yet met with in the water of the Merrimack River at Lawrence. By these means, also, there was isolated from the faeces of a patient sick with a typical case of typhoid fever, at the Lawrence General Hospital, a bacillus which, after long and careful study, appeared to be in every way identical with typhoid cultures obtained from Prudden of New York, and from Chantemesse of Paris. The faeces were passed on the fifth day of the patient's illness. In the course of these investigations eleven examinations of typhoid faeces and three of typhoid urine were made in which the specific organism could not be found.

Of the various other special culture methods and media which have been suggested for the diagnosis of the typhoid bacillus, none have proved to be of sufficient value to warrant them a position in the scheme for the differentiation of this bacillus. This does not necessarily indicate, however, that these special methods and media are valueless when working with waters differing from the Merrimack River in their flora, nor is it claimed that the observations outlined above are sufficient to differentiate the typhoid bacillus from all other species of bacteria. The work of many months goes to show that before any degree of certainty can be reached in the identification of the typhoid bacillus a thorough knowledge must be had of the ordinary species of bacteria present in the source under examination. Until recently the potato test was used to differentiate the bacillus *coli communis* from the typhoid bacillus. At the present time, after careful work by several investigators, it has been found that this test is untrustworthy, and in its place there are, as is well known, three apparently unailing tests, namely: (1) when grown in sterilized milk the bacillus *coli communis* produces coagulation — the typhoid bacillus does not; (2) in sterilized milk the bacillus *coli communis* forms acids — the typhoid bacillus forms a very slight amount or none; (3) in the Smith test the colon bacillus forms a turbidity with gas — the typhoid bacillus a turbidity without gas. (Both bacilli reduce nitrate to nitrite in a peptone solution.)

In obtaining a knowledge of the species of bacteria in the source under examination an opportunity is afforded to ascertain the limits of accuracy of the methods employed. It will also appear if there are any species regularly present which are identical, within the limits of variability, with the typhoid bacillus in all the tests employed. If such species are present then new methods for differentiation must be devised; if they are absent there can be developed, from a classification of the data, a scheme of procedure which will enable the typhoid bacillus to be most readily differen-

tiated. Thus it has been learned after frequent examinations covering considerable more than a year, and involving more than twenty-four hundred species determinations, that there appear to be no other non-liquefying bacteria in the water of the Merrimack River which coagulate milk and also produce a turbidity, without gas, in the Smith test. Accordingly, when a sample of this water is to be examined for the typhoid bacillus, "fishes" are made from the non-liquefying colonies upon the gelatine plate into Smith tubes. Those cultures which produce a turbidity, without gas, are transferred to milk and to the gelatine tube. From the data thus obtained those species which are not typhoid are readily thrown out, while the typhoid bacilli are subsequently passed through all the tests described above and their identity is established.

Much labor can often be saved by the use of the agar instead of the gelatine plate culture grown at 38° C. which is the optimum temperature for the typhoid bacillus but is so high as to inhibit the growth of many water species.

SUMMARY.

(1) After prolonged investigation it has been found that it is possible to separate the typhoid bacillus from all other bacteria hitherto encountered in the water of the Merrimack River.

(2) The potato method of differentiation is of no diagnostic value.

(3) The three tests which have been found to be highly diagnostic are (after non-liquefaction): (1) non-coagulation of milk; (2) non-formation or formation of a very slight amount of acid in milk; (3) production of a turbidity without gas in the Smith test.

In closing, the writer wishes to express his obligations to Prof. W. T. Sedgwick and Mr. Allen Hazen for many valuable suggestions.

BACTERIOLOGICAL AND CLINICAL INVESTIGATIONS INTO THE NEW ANTISEPTIC, DERMATOL.¹

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LAST August, while looking over the *Berliner klin. Wochenschrift*, I saw the statement by Heinz in regard to an antiseptic powder which was to take the place of iodoform. And as the report showed that material, a sub-gallate of bismuth which he called dermatol, had been produced after a long course of experimentation, during which many compounds had been tried, but rejected as unsuitable, I determined to try the dermatol during my dispensary service, and see how my results would coincide with the claims made for it by Heinz. Accordingly I secured a quantity and I began its use about the middle of September, 1891, and continued until January 1, 1892.

During all this time I used the gallate of bismuth in practically all the cases that presented themselves, at the Boston Dispensary, where iodoform, aristol, or a simple, inert powder would have been indicated. During this time there were over 1,300 new patients treated in the surgical department, so that I had abundant opportunity to observe where the new powder was useful, and where it was useless.

I purposely avoided reading any articles which appeared at various times upon the subject, so that I

¹ Read before the Massachusetts Medical Society June 7, 1892, and recommended for publication by the Society.