The Typhoid Bacillus

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Classification of Subject.

Introductory and Historical.
Morphology.
Staining Peculiarities.
Biological Characters.
Pathogenic Properties.
Pathological Effects on Man.
Effects on the Lower Animals.
Methods of Identification.

Introductory and Historical.

The true cause of Typhoid or Enteric Fever has not, as yet, been established beyond question. The symptoms of the disease plainly indicate that it is of bacterial origin, and at different times a number of micro-organisms have been isolated which have been claimed to be the specific cause. However, the bacillus described by Eberth in 1881 is now generally accepted to be the true exciting agent.

This organism was probably observed in 1875, by Browicz, and later (1878) by Fischel, who noticed oval bacteria in sections of the spleen of typhoid cadavers. It was not until 1881, however, that the bacillus was really investigated. Before the announcement of Eberth, Koch had made photomicrographs of sections of the spleen, showing the tissues invaded by the germ.

Gaffky in 1884 was the first to bring forth much evidence to support Eberth's hypothesis. He throly investigated twenty eight cases of typhoid fever and in all but two of these found colonies of the bacillus, in sections of the spleen, liver or kidneys. He states that in all cases of typhoid fever, cultures of the bacillus may be obtained from the spleen. To Gaffky belongs the honor of being the first to obtain a pure culture of the bacillus, and to determine its principal biological character.

Hein, and later Philopowicz, have reported that they were able to obtain pure cultures from material drawn with a hypodermic syringe from the spleens of living patients.

Morphology.

The typhoid bacilli are small rods, having a length, according to Sternburg, of from one to three microns, and a width of five tenths to eight tenths microns. The ends are rounded, and, in young cultures, the bacilli are so short as to almost resemble cocci when examined with a low power objective. In vigorous agar cultures, (three to five days old,) the bacilli appear somewhat shorter than the Bacillus tuberculosis, and are from two, to two and one half times as long as wide. The have somewhat the appearence of Bacillus mallei, but are more oval in form. In old cultures, involution forms are common, in which the bacilli appear as long threads, having about the same diameter as the short rods.

When examined in fresh preparations, the bacilli are seen to be very motile, moving across the field with considerable rapidity. This is more marked in young cultures. When properly stained, the motile organs are seen as long flagella. These are quite numerous and are distributed over the entire surface of the bacterium. They are sometimes as much as five times the length of the rods.

It is extremely probable that the typhoid bacillus does not form spores. While refractive granules have been observed in the bacterial cells, which at first sight appear like spores, they do not show the resistance to heat stains and chemicals, which characterizes true spores. One observer (Buchner) even states that cultures containing these granules are less resistant to destructive agencies than those not containing them.

Staining Peculiarities.

Tho the typhoid bacillus can hardly be said to stain with dificulty, yet it stains much less readily than Bacillus subtilis, or Bacillus anthracis. In the writer's experiments, Water gentian violet was found to yield the best results when preparations could be obtained containing little foreign material. For general work, however, Ziehl's Solution was found most serviceable; the bacilli were not as deeply stained as with the violet, and were not as easily examined with the low power objective, but the fuchsin solution was much better for preparations to be examined with the oil immersion objective. However in certain preparations stained with Ziehl's Solution, the protoplasm of the cell had apparently contracted, giving the bacteria the appearance of streptococci. In a few slides examined, this appearance was so pronounced that the bacteria had entirely lost their characteristic form.

The bacilli were stained by heating with Water Bismarck brown, but a film of the stain was always left on the cover-glass, which injured the appearance of the preparation, and moreover, when mounted in balsam, the bacteria seemed to lose some of their color.

Malachite Green (Alcoholic water solution and aniline water solution) and Methylene Blue (Acid solution and water solution) were tried, but with unfavorable results.

The writer attempted to show the flagella, using a slight modification of Van Ermengen's method, but without success. The flagella are said to stain by Loeffler's method if the mordant be made slightly alkaline.

Biological Characters.

Eberth's bacillus is a facultative anerobe, although it grows best in the presence of free oxygen. It grows readily on most culture media, and at the ordinary room temperature, but better in the incubator at a temperature of from 37° to 40° C.

On agar thrust cultures in the incubator, the track of the needle is distinctly seen in twelve hours, and in twenty four hours a colony or group of colonies on the two millimeters in diameter, has formed at the surface. This latter growth rapidly increases, until, at the end of two or three days, the entire surface is covered. The colonies are rather irregular in outline and are of a transparent, whitish color. At the end of three or four days, the centre of the colony begins to take on a brickred appearance, and this gradually spreads toward the periphery. The culture has a peculiar, but not unpleasant odor.

On potato, the growth has at first the form of a shining white layer, the so called "invisible growth". From the naked-eye appearance of the potato, it would probably not be suspected that there was any growth on it; but if a little material be taken from near the site of inoculation, it will be found to consist of almost pure bacilli, mixed with very little foreign material. After about two days, the growth becomes visible as a brown spot, increasing in depth and area. It was formerly thought that this mode of growth on potato was peculiar to Eberth's bacillus, but it has since been shown that this is not the case. It is, however, an important character for the differentiation of the germ from Bacillus coli commune, and other bacilli found in the intestines.

The growth on gelatin is somewhat similar to that on agar. It does not liquify it. On beef bouillon, after some days, the culture shows a copious white precipitate.

The typhoid bacillus is said to flourish in culture media containing amounts of phenol, that would be fatal to almost all other bacteria. A number of experimenters have applied this property and claim that they are able to obtain the bacillus in a pure culture, by adding from $\frac{15}{100}$ % to $\frac{20}{100}$ % phenol to the nutrient medium.

Eberth's bacillus does not produce indol, a fact which is sometimes made use of in diagnosis.

The bacillus thrives readily in almost any nutrient medium, and, under favorable conditions, it multiplies with great rapidity. This can be seen to have an important bearing on sanitation. If the excretions and discharges of typhoid patients are not disinfected, the adaptability of the microbe to saprophitic conditions enables it to maintain its existence for long periods of time, in the soil, from which it may pass to wells, etc. Milk, contaminated with the germ, would; in a few hours become highly dangerous.

Pathogenic Properties

Pathological Effects on Man.

Typhoid fever, the disease supposed to be due to Eberth's bacillus, is an inflammation and ulceration of the intestines, accompanied by high fever and other constitutional symptoms.

The ileum is the chief part affected, tho the colon, and more rarely the jejunum, duodenum and stomach may be the seats of lesions. The follicular glands in the lower end of the ileum become infiltrated with small, round cells and, as a result, in from ten to fourteen days they slough out and leave ulcers. The gland tissue has become dead and is detached in shreddy masses. If the intestinal wall has not been perforated

the serous coat of the bowel is little changed.
In case of Peyer's Patches, the disintegration of the bowel may go so far as to cause perforation and thus occasion an abscess adjacent to the point of perforation, or, the escape of fetal matter may cause death from peritonitis. The ulcers of the isolated glands do not show the same tendency to penetrate deeply.

In most cases of typhoid fever, the mesenteric glands are swollen, and sometimes almost all of the lymphatic glands, as well as the tonsils, and base of the tongue are enlarged. The spleen is nearly always affected, being enlarged in size, and containing the bacillus in almost a pure culture. The liver and kidneys are also often enlarged.

Effects on the Lower Animals.

Eberth's bacillus appears to be unable to multiply in the bodies of the lower animals. Gaffky was unable to induce typhoid symptoms in apes, rabbits, guinea pigs, mice, rats, or pigeons, by feeding these animals with pure cultures of the germ. Tho the lower animals are thus apparently immune to typhoid fever, they may be killed if inoculated with a sufficient quantity of a culture. The amount necessary to produce death may be very small. The results obtained are similar when the animal has been inoculated with a culture containing the living germs, and when a culture sterilized by heat or filtration is used. It therefore seems evident that the pathogenic power (for the lower animals) of these cultures is due to a specific poison or toxin formed by the bacillus in the culture media, and not to its multiplycation in the body of the animal. Brieger claims to have isolated this toxin, and describes it as a very deliquescent substance, having strongly basic properties. He has named it "Typhotoxin", and ascribes to it, the formula $C_7H_{17}NO_2$. In guinea pigs, small doses of this substance produced salivation, then paralysis, and later, dilated pupils and death. Diarrhoea accompanied all these symptoms.

Methods of Identifycation.

Owing to the fact that there are many

other bacteria found in water and soil, which are almost identical with Eberth's bacillus, in their biological and morphological characters, it is extremely dificult to identify it when found in drinking water, sewage, etc.

Moreover, as none of the lower animals are susceptible, inoculation experiments cannot be tried. The following methods have been recommended by different bacteriologists, for its detection or identification.

Parietti's Method.

A solution is made as follows:—

Carbolic Acid	5 grams.
Hydrochloric Acid	4 grams.
Water	100 C.C.

From three to nine drops of this solution, is added to each of several tubes, which contain ten cubic centimeters neutral, sterile bouillon each. The tubes are then placed in the incubator twenty four hours, and, if still sterile, from one to ten drops of the suspected water is added to each tube. They are then returned to the incubator. If, at the end of twenty four hours, a cloudiness should appear, this is due, according to Parietti to the presence of the

Typhoid bacillus.

Another method is to make plate cultures of the suspected water and keep them at a temperature of 40° C, in the incubator. As most water bacilli do not thrive at this temperature, any typhoid bacilli present, will form colonies which may be recognized, and the germ obtained in pure culture.

Typhoid bacilli may be distinguished from many similar bacteria:—

(1.) By their growth on potato.
(2.) By the absence of indol in pure cultures
(3.) By the fact that they produce no gas in nutrient media containing sugar.

None of these are sufficient, however, to identify the bacillus when used alone. Even by the use of all, reliable results can be obtained only by a person of some experience.