

The Bacteriological Examination of Water.*

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During the last few years the literature of the subject of Bacteriology has become so extensive that anything like a general epitome of the subject is quite beyond the scope of a paper for a single evening. I shall, therefore, restrict my remarks to that department of the subject which has a practical application in the examination of water for drinking purposes.

Before proceeding to the subject proper, it may, however, be well to give a general idea of the characters of the micro-organisms under consideration, so that those who are not already familiar with them may be able to understand more clearly the facts referred to. Those organisms commonly grouped together under the term "bacteria" are unicellular bodies of extremely minute proportions; some idea of their size may be given by the statements that if 10,000 of some of the smaller forms were placed end to end the thread would be only one inch long, and a cubic inch of the organisms in mass would contain ten millions of millions of individuals. These organisms are not all of the same form, and, in consequence, may be arranged into various classes; perhaps Cohn's system is the most convenient for general use:

(1) *Coccaceæ*. Ball bacteria. Cells spherical or shortly elliptical.

(2) *Bacteriaceæ*. Rod-bacteria. Cells distinctly longer than broad, straight rods, varying in length,

(3) *Spiro-bacteria*, *Vibrionaceæ*. Cork-screw bacteria. Cells curved, often twisted into long or short spirals.

(4) *Desmo-bacteria*. Thread bacteria. Cells united to form long threads, some being enclosed in sheaths.

The bacterium consists of protoplasm enclosed in a membrane, usually of an albuminous nature. Some species possess flagella, by means of which they have the power of motion, and which may be situated on certain parts of the cell only, or distributed over the whole surface. In some species no flagella have been observed. Of the motile forms some species may be seen to dart rapidly across the field of vision, others move but sluggishly.

Some forms develop distinctive colors, but the majority are colorless or yellowish.

A fact of the utmost importance is that bacteria are unable to rise in air, unless carried upward by a draught, and in water, at rest, most species subside to the bottom.

Many kinds of bacteria produce spores, which are far more resistant to the effects of temperature, etc., than the parent bacteria; these spores often remain as such for a considerable period of time, and then develop when favorable conditions obtain.

The application of bacteriology to the subject of water supply may conveniently be considered under three sections:

(1) The determination of the efficiency of filters.

(2) The general examination of water as to suitability for drinking purposes.

(3) The special examination of polluted water for individual species of bacilli.

1. THE DETERMINATION OF THE EFFICIENCY OF FILTERS.

The value of bacteriological examination for this purpose has for several years past been recognized, and the system is now in full operation in connection with the water supply of many towns.

For this purpose samples of water must be taken before and after filtration—in such a position that the samples shall fairly represent the water actually running at the time—and the number of bacilli in each sample determined. The bottles in which the samples are taken must be previously sterilized, either by heating for three hours to a temperature of 130°–160° C., or by filling with a solution of bichloride of mercury (1–1,000), allowing to stand one hour, and then rinsing six or eight times with water previously rendered sterile by boiling for an hour in a flask plugged with cotton-wool, and covered with a small, sterilized beaker. Water may also be sterilized by passing through a Chamberland-Pasteur or a Berkefeld filter. The stoppers of the sterilized bottles are tied over with sterilized gutta-percha tissue, and transported in tins or other suitable containers.

When taking the samples, every care must be exercised to avoid the possibility of infection from any external source; the stopper must be removed by grasping between finger and thumb, together with the gutta-percha capping, and not laid down upon the ground, but held until the sample has been taken; the bottle is held in the other hand in such a position that no bacteria from the skin can enter the bottle; this is easily arranged when the supply is a flowing stream, but if a pool or reservoir the bottle should be held by a sterile clamp. The neck of the bottle must be completely immersed, so as to avoid the entrance of any dust which may have settled upon the surface of the water. When full, the stopper is replaced, and tied over as before. If the supply is carried by a pipe, the tap should be turned on for several minutes before taking the sample.

When the test has to be carried out at a distance, it is needful to pack the tins in ice, so that the temperature may not rise above 4° or 5° C. by the time the test is made. The case shown is one in constant use, the temperature twenty-four hours after packing is 0° C. The reason why it is so important to keep down the temperature is that the organisms multiply extremely rapidly at ordinary temperatures, but very slowly, or not at all, when kept at or near 0° C. Unfortunately,

however, some species of bacteria are killed at the freezing point, consequently the results obtained may be lower than the truth. Moreover, the development of those which are not killed is much retarded by long continued low temperature. The bacteria in pure water obtained from deep springs usually multiply far more rapidly than those from streams; this is no doubt owing to the fact that the former waters do not contain any of those products of bacterial life which inhibit their further growth, whereas the latter usually do contain such products, and living bacteria in far larger numbers in addition. As an instance of rapid multiplication, Frankland gives the following figures:

Kent Well. On day of collection. 7 bacteria.
 " After 1 day at 20° C. 21 "
 " After 3 days at 20° C. 495,000 "

The multiplication usually attains a maximum by about the seventh day, in the case of spring waters, but not until the fifteenth to twenty-fifth day in impure water of streams, after which the numbers rapidly decline.

The number of bacteria is ascertained by introducing small portions of the water into a suitable nutrient, and counting the colonies developed. This is commonly carried out in small flat-bottomed glass dishes known as Petri's dishes, which are furnished with covers of the same form. The most convenient medium for cultivation is gelatine-peptone, because this is solid at the ordinary temperature, yet may be liquefied by a very gentle heat, and thus easily and uniformly mixed with the water under examination. For its preparation a pound of lean minced beef is infused with a litre of cold water, and allowed to stand for twenty-four hours in a cold place, then strained and pressed, adding water to the strained liquor to produce one litre, if needed. To this liquid are added 100 grammes of fine white gelatine, 10 grammes of dry peptone, and 5 grammes of salt, and the whole placed in a steam sterilizer for an hour, after which it must be carefully neutralized, or rather rendered faintly alkaline. As the degree of alkalinity has a most important influence upon the growth of bacteria, the best plan is to remove a small portion of the medium and titrate by means of $\frac{N}{10}$ sodium hydrate, using phenol-phthalein as an indicator; having in this way ascertained the amount required for exact neutralization the correct quantity of normal sodium hydrate is added together with 1 gramme (per litre) of crystalline sodium carbonate; this being the degree of alkalinity which gives the best results. To this liquor, when cool, the whites of two eggs are added, and after admixture the whole is placed in the steamer for twenty minutes, when the coagulated albumen is removed by straining through linen, and finally cleared by filtration through white filter paper at a temperature of about 45° C. The filtrate is collected in a flask which is plugged with cotton-wool, previously sterilized at 130° to 140° C. trans-

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