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THE VALUE OF A BACTERIOLOGICAL EXAMINATION OF WATER FROM A SANITARY POINT OF VIEW.[1]

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SEVERAL years ago, when connected with the Massachusetts State Board of Health, the author had occasion to make bacteriological examinations of a large number of samples of water, derived from various sources. The method then in use was to mix one cc. of the water with about nine cc. of sterile nutrient gelatin, and to pour the mixture upon sterilized glass plates, where it solidified in a moderately thin layer. These plates were then kept under cover for two, three, or four days, to protect them from dust and from drying, after which they were examined either under the microscope, with a hand lens, or with the unaided eye. The number of colonies that developed on these plates was then recorded.

It appeared to me at that time, 1887, that it was not possible to judge of the quality of a water from the data so obtained. This opinion was supported in a striking manner by observations, of which the following is a good, though somewhat extreme, example. The water supply of one of the towns near Boston was derived from a series of driven wells, consisting of iron pipes forced into the sandy soil. All but one of these wells were connected with a pipe leading to the pump which drove the water into the town reservoir. The remaining well was not connected with the others, but was used for making observations

[1] Read at the Special Meeting of the New York Section, April 23, 1897.

on the level of the ground water. It was kept covered, except when used for that purpose.

I found that the water taken from the tap at the pumping station, after it had passed through the pump, contained two bacteria to the cubic centimeter, *i. e.*, it was practically sterile. Water taken from the observation well contained about 5,000 bacteria to the cubic centimeter.

The water in both cases was from the same source, namely the ground water, but that which passed through the closed pipes was protected from infection, while that from the observation well had been exposed to the entrance of bacteria from the air. The bacteria found in the water from this well were all of the same species, and the inference was plain that, of those bacteria which accidentally gained access to the water, only one species had found the conditions favorable for its indefinite multiplication. There could not be any doubt that the two waters were equally fit for drinking, provided the single species, which was present in such abundance, was unobjectionable. At that time the maximum number of bacteria that was considered permissible in a good water was set at 250; yet this perfectly wholesome water contained twenty times that number.

Similar observations soon led me to the conclusion that a water might contain a large variety of bacterial species without being injurious to health. The water of small brooks flowing from springs in wooded regions frequently contains numerous species of bacteria derived from the air and the banks of the stream, yet there is no reason to suppose that such water is unfit for drinking.

The manner, then in vogue, of interpreting the results of such a bacteriological examination by the plate method appeared to me to be artificial and inadequate, because the accepted standards of purity were based upon quantitative differences. The method itself was not wholly without value, for it appeared possible to gain some insight into the probable bacterial history of a water, by a simple inspection of the plate cultures. prepared from it. Pure waters, originally free from bacteria, but subsequently exposed to the atmosphere, when examined by plate culture, yielded colonies composed of bacteria, which, as a rule, failed to liquefy gelatin and frequently possessed chromogenic

powers. The water accumulating in collecting wells or trenches protected from surface drainage by walls of brick or stone and covered by structures permitting free ventilation, gave plate cultures of this character. The bacteria it contained were derived from the air.

Those waters which had received additions coming in contact with vegetation and the upper strata of the soil, contained the bacillus subtilis, the bacillus mycoides, and the bacillus figurans, all easily recognizable by the characters of the colonies resulting from their growth on gelatin plates. The waters of unpolluted brooks and small streams arising from springs in rural districts contained these bacteria, as well as those coming from the air.

It is doubtful whether any of the waters from larger streams or rivers, which I examined, were free from pollution by either sewage or waste from factories. The plate cultures from such waters revealed a varied flora, and it was not possible to identify, on the plate cultures, the species that I regarded as evidence of the presence of air bacteria and bacteria derived from the soil. It is probable that the bacteria resulting from the pollution of the water were more abundant or grew more rapidly than those from the air and soil and, in consequence, concealed their presence.

Plate cultures made from sewage and those prepared from waters that had certainly received sewage, contained colonies of a yellowish or brownish color, when viewed under the microscope. They were round, oval or lenticular in shape, and usually presented a multicontoured appearance, if the plates were not too crowded. These colonies I now believe to have been those of the common colon bacillus. The practically constant presence of such colonies, on plates from water contaminated with sewage, led me to look upon them as an indication of dangerous pollution.

I think there were good grounds for the opinion I then formed that the mere number and variety of bacteria in a water are of comparatively little significance, but that the presence of certain species might be accepted as indicating that the water had been exposed to certain pretty definite conditions before it was submitted to bacteriological examination, and that a knowledge

of those conditions was of importance to a just estimation of the probable risk of drinking the water.

The chief aim of an examination of water with reference to its fitness for drinking is to learn whether it contains, or is likely in the future to contain, poisonous substances, or the contagia of disease. The detection of mineral poisons falls within the domain of chemistry. The actual isolation of specific bacteria of disease can only be done by bacteriological methods. But in the vast majority of the cases in which an examination of water is required, mineral poisons are not present in notable quantities, nor are pathogenic bacteria so abundant as to be certainly detected by the methods now available. The object of an examination of the water in these cases is to estimate the degree of probability that in some future time the particular water in question may become the carrier of infection. This question practically resolves itself into the detection of pollution with sewage, which may at some time contain dejecta from cases of disease. The usual chemical examination aims at detecting such pollution, and a bacteriological examination may be directed towards the same end. By the former method, the soluble substances, which are abundant in sewage, are estimated. By the latter, the bacteria derived from the intestinal tract and those of putrefaction, which thrive in solutions rich in animal organic matter, are sought, and, if need be, isolated. When sewage is mixed with water, both these methods for its detection are applicable, but it appears to me that the bacteriological test is capable of being the more delicate and precise of the two methods.

An exhaustive bacteriological examination which undertook the isolation of every species present in a given water, and endeavored to trace the way in which each species gained access to the water, would require a much wider and more exact knowledge of the distribution of bacterial species than we at present possess. And even if that knowledge were available, such an examination would require a very protracted study of the water. It appears necessary, therefore, to resort to a few simple procedures which are likely to give the information strictly required for a just conception of the general bacterial

history of the water and to base the judgment of the sanitary value of the water upon the results so obtained.

I will give a brief outline of the methods that appear to me most likely to furnish useful knowledge respecting the wholesomeness of a water from a bacteriological standpoint, assuming that the main questions to be answered are: (1) whether the water has been polluted with sewage, and (2) in case there are many bacteria in the water, whence they were probably derived.

It so happens that most of the bacteria found in the air are strict aerobes, *i. e.*, are incapable of growth, save in the presence of oxygen. We may take advantage of this fact to gain a rough idea of the number of bacteria in the water which owe their presence to aerial contamination. My plan is to prepare four gelatin plates, with one cc. of water on each plate, and to allow two of them to develop in an ordinary moist chamber, the other two being kept in an atmosphere of hydrogen. When those which have been exposed to oxygen are ready for counting, all four plates are examined and the number of colonies estimated. I will give examples of the two sets of observations, illustrating the results obtained by this method.

Experiment I.—Distilled water taken from a demijohn which had been exposed to contamination from the air for at least two weeks, was examined in this way. At the end of two days the plates grown with access of air contained 1,395 colonies. Those grown in hydrogen showed no colonies at all. The latter were then placed in a moist chamber, where they were exposed to oxygen for two days. At the end of this time they contained 1,469 colonies, showing that the bacteria in the water were not *killed* by the hydrogen, but were incapable of growing to any great extent when in an atmosphere of that gas.

Experiment II.—A similar experiment was made with croton water. The plates grown in air contained 135 colonies. Those grown in hydrogen contained 30 colonies, but, after exposure to the air, the number increased to 128 colonies. It seems, therefore, that the croton water contains species that were at least facultative anaerobes, and probably some that were not originally derived from the air.

Those bacteria that are capable of producing specific diseases are nearly all facultative anaerobes, so that it is safe to infer

that the distilled water taken for the first experiment was fit for drinking.

It is probable, from their microscopical appearance, that some of the colonies on the hydrogen plates from the croton water, were those of the proteus vulgaris, one of the bacteria most frequently found in putrefying organic matter, of animal origin.

Although the bacteria, prevalent in the air, are usually dependent upon the presence of oxygen for their multiplication, it must not be assumed that all the strictly aerobic bacteria, found in a water, have been derived from the air. Many of those occurring in the soil are incapable of growing without a supply of free oxygen. This is shown by the following experiment.

Experiment III.—One gram of soil from near the surface of the ground was introduced into a liter of water which had been sterilized by prolonged boiling, and after vigorous shaking, plates were prepared as in the preceding experiments. The plates grown in air contained 38,771 colonies, those grown in hydrogen 534 colonies. The general appearance of the two sets of plates was very different. The air plates contained numerous colonies of mycoides, figurans, and the hay bacillus. The hydrogen plates contained only a slight growth of figurans, visible under the microscope. The hay bacillus and mycoides did not develop on the plates grown in hydrogen.

If we now turn our attention to the results obtained by this method, when sewage is examined, we shall find that the number of facultative bacteria is very much greater in proportion to the number of strict aerobes, than was the case in the experiments with water, contaminated with species from the air or soil.

Experiment IV.—Plates were prepared, each with one cc. of sewage from one of the main sewers of this city. After twenty-four hours the colonies were estimated with the aid of the microscope. The air plates contained an average of 51,516 colonies, the hydrogen plates 49,871, a difference of only 645 in over 50,000.

Experiment V.—One cc. of the same sewage was added to one liter of sterilized distilled water, and with one cc. each, the plates were prepared. Those grown in air contained 260 colonies, those grown in hydrogen 278. In this case the number of colo-

nies on the hydrogen plates was greater than the number on the air plates, a circumstance that need occasion no surprise when we reflect that the sewage contained little masses of suspended matter that would prevent a perfectly uniform mixture with water, and might, therefore, easily cause some of the samples, taken for bacteriological examination, to contain more bacteria than others.

Experiment VI.—The diluted sewage used for the last experiment was allowed to stand at the room temperature for three days and the examination was then repeated. The plates grown in air contained 18,187 colonies; those grown in hydrogen 17,197.

The last three experiments show that a large proportion of the bacteria in sewage are probably facultative anaerobes, and that a considerable dilution of the sewage does not prevent a rapid multiplication of the bacteria it contains. Such a dilution would improve the chemical character of the water, but leaves its bacteriological character unchanged.

A comparison of the results of the six experiments, detailed above, demonstrates that the method used is capable of throwing considerable light on the significance of the bacteria found in a sample of water.

It would not do, however, to leave the consideration of that method without pointing out a possible source of error in the deductions, drawn from the results of these experiments. It might be that there were two sets of bacteria on the plates, one consisting of strict aerobes, and the other of strict anaerobes, and that one or the other of these groups would develop on the plates, according to whether they were exposed to oxygen or not. I believe myself justified in excluding this possibility, on the ground that the characters of the colonies on the hydrogen plates were essentially the same as some of those on the plates grown in the air, both when examined under the microscope and with the unaided eye. The fact that the hydrogen plates, when subsequently exposed to oxygen, contained practically the same number of colonies as those which were originally grown in the presence of oxygen, also tends to exclude the source of error we are considering.

The simple procedure used in the above experiments would not alone suffice to reveal the presence of sewage. We must

gain a more definite idea of the characters of the bacteria in the water, where it is shown to contain facultative anaerobes before we are justified in concluding that they are an indication of sewage contamination.

All sewage that receives human feces contains the bacillus coli communis, or if it does not, has been subjected to germicidal agencies that would also kill pathogenic bacteria, derived from cases of disease. It is fair to assume that ordinary sewage would also contain the common bacteria of putrefaction. We must, therefore, direct our attention to the means of demonstrating the presence or absence of those species in the water under examination.

I am inclined to believe that the best way to accomplish this is the application of the putrefactive test based upon the method proposed by Schardinger in the *Centralblatt für Bacteriologie und Parasitenkunde*, 16, 833, *1894*.

To about ninety cc. of the water, ten cc. of a ten per cent. pepton, five per cent. salt solution, previously sterilized, are added. The mixture is made in a sterile Erlenmeyer flask, provided with a cotton plug. A strip of paper, impregnated with lead carbonate, is suspended over the mixture and the flask is then placed in the incubator at 37° C. for twenty-four hours. Under these conditions of temperature and nutrition, the colon bacillus and the bacteria of putrefaction readily multiply, and the latter cause the production of hydrogen sulphide, which discolors the lead paper. A pure water will not cause a darkening of the paper, but as it is possible that a water which would not cause infection, either at the time or in the future, might contain putrefactive bacteria, this test alone should not be relied upon to decide whether a water is fit for drinking or not.

In order to detect the presence of the colon bacillus a loopful of the above mixture, after the twenty-four hours of incubation, may be used for the preparation of a series of plate cultures in various degrees of dilution. From these plates there is no difficulty in obtaining pure cultures of that bacillus, which may be used for further cultures made for the purpose of definitely identifying it, and, especially, of distinguishing it from the bacillus equi intestinalis, which appears to be the most common species present in the feces of horses.

This method takes considerable time, and if it be desired to simply prove the absence of the colon bacillus, there is a shorter method which can be employed. It consists in inoculating a series of fermentation tubes, containing nutrient bouillon, to which two per cent. of glucose has been added, with the water under examination, using one cc. of water for each fermentation tube. The tubes are then placed in the incubator. The colon bacillus has the power of causing fermentation with a production of gas when grown in sugar solutions. This gas collects in the upright limb of the fermentation tubes. If no gas is found in any of the tubes, it may be assumed that the colon bacillus was absent. But if gas is found it does not prove that the colon bacillus was present, for other bacteria are capable of decomposing sugar with a production of gas. It then becomes necessary to isolate the bacteria in the fermentation tube with a view to determining the presence of the colon bacillus, a matter of no difficulty.

The procedures which are now outlined constitute those which are thought most likely to throw light upon the sanitary value of a water. Let me next call attention to the results obtained by their employment in the examination of some samples of water.

Experiment VII.—The results obtained from croton water were as follows:

1. Plate cultures grown in air contained 135 colonies.
2. Plates grown in hydrogen contained thirty colonies.
3. The putrefactive test blackened lead paper within twenty-four hours, and emitted a foul, somewhat fecal odor. Plate cultures from the putrefaction flask revealed the presence of the colon bacillus and the bacillus proteus vulgaris.
4. The fermentation test showed a production of gas, and cultures made from the fermentation tubes revealed the colon bacillus.

It seems safe to infer from these results that the croton water had been exposed to pollution with sewage, but that the latter had been greatly diluted, and probably also exposed to contamination from the air and admixture with some surface drainage. The latter inferences are based upon the large proportion of

aerobic bacteria in the water, and of colonies of mycoides and subtilis on the plates developed in the air.

Experiment VIII.—A reservoir water from a town near New York yielded the following results:

1. Plates grown in air contained 2,090 colonies.
2. Plates grown in hydrogen contained 165 colonies.
3. The putrefactive test caused no blackening of the lead paper.
4. The fermentation test showed the production of gas, but cultures prepared from the fermentation tubes failed to reveal the presence of the colon bacillus. There were a few colonies of mycoides on the plates grown in air.

The evidence furnished by these results are in accord with the known facts about this water. They permit the inference that the water had not been contaminated with sewage, and was therefore free from putrefactive and intestinal bacteria, but that it had been exposed to the air and to contact with the upper layer of the soil.

If we accepted the simple enumeration of the bacteria in a water as a guide to a judgment of its purity, we should be erroneously led to consider croton water as purer than this reservoir water, whereas the methods actually employed show the reverse to be the case.

Where the presence of sewage and surface drainage is revealed by these methods, as in the case of the croton water mentioned, we should, in estimating the risk of infection from drinking the water, consider (1) the extent to which the sewage has been diluted, and (2) the dangers incident to the surface drainage.

That the dilution had been considerable is shown by the bacteriological examination, for the number of anaerobic bacteria was small, but the degree of dilution cannot be so accurately gauged by that method as by a chemical examination. Croton water, at the time this bacteriological examination was made, revealed nothing, on chemical examination, which would cause suspicion that it contained sewage. The dilution must, therefore, have been very considerable.

The danger from the surface drainage could only be estimated by a local inspection of the surroundings of the water.

The results of these examinations and the inferences drawn from them are in close agreement with the known facts regarding the croton and reservoir waters, and they tend to confirm the value of the procedures described.

But it does not always happen that the results of chemical and bacteriological examinations are in such close accord. I had occasion at one time to examine the water from a deep artesian well, and found the water practically free from bacteria. The chemical examination showed the presence of large quantities of the ammonias, nitrates, and chlorides, and the chemist's report strongly condemns the water. Local inspection revealed the fact that the fields around the well were used for the disposal of the sewage from a large penal institution in the neighborhood, and that the water from the well was used to supply that institution.

It appears to me, that under these conditions, which had persisted for years, the water might be considered as free from objections so long as the upper portions of the well remained water-tight. For it was evident that the percolation of the sewage through the soil removed the bacteria which it contained. The three modes of investigation reveal the conditions obtaining at the time and also point out the possible future dangers.

It would not do to regard the presence of the colon bacillus as a proof of pollution with sewage without other confirmative evidence. The feces of cattle and other animals contain that bacterium, and it is a pretty widely distributed saprophyte, especially in inhabited regions. The fact that it is found in milk, and, after a day or two, in the intestinal discharges of new-born children, is often cited as proof of its wide distribution. I do not think that too much stress should be given to that evidence when we consider the way in which children are born, and the way in which cows are usually milked. If the colon bacillus is present in a water in considerable quantities, as shown by a series of fermentation tubes, it is very unlikely that it gained access to the water in an innocent way.

When the colon bacillus reaches a water in company with sewage, there is sufficient organic matter of animal origin to furnish it with nourishment, favoring its rapid multiplication. We have had an example of this multiplication of the sewage

bacteria, of which the colon bacillus formed a considerable proportion in Experiment VI. In three days the number of bacteria rose from 260 to 18,187, an increase of nearly 600 per cent.

In order to gain a clearer idea of the conditions under which the colon bacillus would multiply in water, the following three experiments were instituted:

Three sterilized, cotton-plugged flasks each received one liter of distilled water, which was boiled in the flasks for one hour to sterilize it.

Experiment IX.—To one of these flasks one cc. of a filtered suspension of the colon bacillus, in sterilized distilled water, was added. The bacillus had been grown upon agar in order to get an abundant growth with the least admixture of organic matter from the nutrient medium. It was then carefully scraped off the surface of the agar and mixed with the sterilized water. After this the mixture was filtered in order to remove any considerable masses of bacilli not broken up during the mixing. After vigorous shaking the water in the flask contained 42,791 bacilli per cubic centimeter, as shown by plate culture.

The flask was kept at the room temperature, exposed to diffuse daylight. After twenty-four hours the water was again examined, when it was found that the number of bacilli had fallen to fourteen.

Experiment X.—The second flask received, in addition to the colon bacillus, one cc. of nutrient bouillon. After the mixture was made, the water in this flask contained 57,102 bacilli to the cubic centimeter. After twenty-four hours it contained 29,276.

Experiment XI.—The third flask received an addition of one cc. of an infusion of hay, instead of the bouillon. On the first day the water contained 14,030 bacilli. After twenty-four hours the number had fallen to 439.

These three experiments go to show that the colon bacillus requires a considerable quantity of organic matter for its abundant multiplication. Dr. A. P. Hallock kindly made a chemical examination of the water in these three flasks, with the following results:

PARTS IN 100,000.

Flask 1.	Free ammonia	0.00575
	Albuminoid ammonia	0.00175
Flask 2.	Free ammonia	0.03675
	Albuminoid ammonia	0.2672
Flask 3.	Free ammonia	0.0125
	Albuminoid ammonia	0.0008

Numerous experiments have been made by various investigators to learn the behavior of the colon bacillus when introduced into natural waters, both with and without previous sterilization. Their results have, in the main, been the same as those just given for these artificial mixtures. As a rule the number of bacilli decreased, but sometimes it remained about the same, or increased slightly, but not to the same degree as did the number of so-called "water bacteria" in the sample.

It seems to me that these observations support the belief that the colon bacilli, which accidentally gained entrance to a water, without an associated pollution with sewage, would fail to multiply to any great extent, and that they would certainly very rarely lead to erroneous inferences from the results of a bacteriological examination. Neither the colon bacillus nor the widely distributed proteus vulgaris cause the evolution of hydrogen sulphide when they are present in water submitted to the putrefactive test. So that an accidental presence of those bacteria would not be bacteriologically equivalent to admixture with sewage.

In this connection it would be of interest to know about the viability of the colon bacillus when subjected to desiccation and light.

The following experiments have a bearing upon this question:

Small threads of sterilized silk were moistened with bouillon cultures of the colon bacillus and then dried in a desiccator over sulphuric acid.

Experiment XII.—Bouillon culture grown in the incubator. Threads exposed to diffuse daylight. The bacillus failed to develop when the threads were placed in fresh bouillon after thirteen days of desiccation.

Experiment XIII.—Bouillon culture grown at the room temperature. Threads exposed to diffuse daylight. Bacillus dead after twenty days.

Experiment XIV.— Bouillon culture grown in incubator. Threads exposed to sunlight. Bacilli dead after six days.

Experiment XV.—Bouillon culture grown at the room temperature. Threads exposed to sunlight. Bacilli dead after ten days.

Similar experiments made with threads moistened with a suspension of human feces, gave the following results:

Experiment XVI.—When exposed to drying over sulphuric acid *in vacuo*, or under ordinary atmospheric pressure, and when exposed to diffuse daylight or direct sunlight, these threads all produced a distinct growth in bouillon after thirty-four days, when the observations were discontinued.

It therefore appears that the viability of the colon bacillus depends upon the conditions under which it is placed. There is apparently no danger of its speedy death when it is associated with fecal matter.

Experience shows that many natural waters, which might readily have received small quantities of the colon bacillus from dust or the surface of the ground, do not contain it in sufficient abundance for its detection by the methods described. This experience is in harmony with the results of the experiments here recorded.

It is, of course, conceivable that a good water might contain adventitious colon bacilli, and this possibility should be borne in mind when conclusions are drawn from the results of a bacteriological examination. It is not probable, however, that such a water would contain a large proportion of anaerobic bacteria, or give a positive outcome to the putrefactive test. If the colon bacillus appeared in considerable quantities in a water, as the result, for example, of drainage from barn-yards, it seems to me that it would indicate an objectionable pollution, even though it were no sign of the presence of human excreta. The author believes, therefore, that the possible conditions which might lead to erroneous inferences from the results of the bacteriological methods of water examination here described, do not seriously invalidate the conclusion that the plan offered is better calculated to give a just estimate of the fitness of a water for drinking purposes than the methods in more common use, and

that they are likely to be of considerable service in a sanitary examination of water.

It is hoped that those who are interested in the subject will put these methods to a practical test in order that their true value may be ascertained, through the experience resulting from their extensive employment.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE U. S. DEPARTMENT OF AGRICULTURE. NO. 29.]

ON THE INFLUENCE OF VEGETABLE MOLD ON THE NITROGENOUS CONTENT OF OATS.

BY H. W. WILEY.

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IN growing oats in pots containing vegetable soils from Florida, I noticed that the content of nitrogen in the product was much greater than in oats grown in common soils under the same cultural and climatic conditions. Previous to the beginning of the experiments described below I had noticed a peculiar condition of sugar-cane grown in Florida, on what is there known as muck soils. These soils are composed of vegetable mold, produced under the water in shallow lakes and along the banks of streams. A full description of these soils may be found in my paper on the "Muck Soils of Florida," published in Agricultural Science, 7, 106.

The condition referred to in the sugar-cane was manifested by a brownish color in the juice, which was extremely persistent, affecting even the color of the nearly pure sugar made therefrom. From the character of this coloration there is no doubt of its being due to an actual absorption by the growing sugar-cane of some of the components of the vegetable soil or humus. The fact that plants, under certain conditions, have the faculty of absorbing humus has been subsequently confirmed by the experiments of Snyder.[1] The vegetable soils in which the oats were grown contain in the air-dried state over eighty per cent. of organic matter and less than ten per cent. of sand and other mineral ingredients. The composition of four samples is shown in the following table:

[1] Bull. No. 41, Agricultural Experiment Station of Minnesota.