THE SIGNIFICANCE OF BACTERIOLOGICAL METHODS IN SANITARY WATER ANALYSIS.*

By C. E. A. WINSLOW and C. P. NIBECKER.

1. *Sanitary Inspection and Its Limitations.*—It is well recognized to-day that a study of the possible sources of pollution on a watershed, of the direction and velocity of currents above and below ground, of the character of soil, and the liability of contamination by surface wash, may furnish the expert with evidence of the greatest value. Often, however, he must form some opinion upon the quality of water sent from a distance without the opportunity of examining its surroundings; and even when a sanitary inspection can be made its results are by no means conclusive. No reconnaissance can show certainly whether unpurified drainage from a cesspool does or does not reach a given well; whether sewage discharged into a lake does or does not find its way to a neighbouring intake; whether pollution of a stream has or has not been removed by a certain period of flow. Evidence upon these points must be obtained from a careful study of the characteristics of the water in question, and this study can be carried out along two lines, chemical and bacteriological.

2. *The Significance of the Chemical Analysis.*—A chemical examination of water for sanitary purposes is mainly useful in throwing light upon one point—the amount of decomposing organic matter present. Humus-like substances may be abundant in surface waters quite free from harmful pollution, but these are stable compounds. Easily decomposable bodies, on the other hand, must obviously have been recently introduced into the water, and mark a transitional state. “The state of change is the state of danger,” as Dr. T. M. Drown has phrased it. Sometimes the organic matter has been washed in by rain from the surface of the ground; sometimes it has been introduced in the concentrated form of sewage. In any case, it is a warning of possible pollution, and the determination of free ammonia, nitrites, carbonaceous matter as shown by “oxygen consumed,” and dissolved oxygen, yield important evidence as to the sanitary quality of a water.

Furthermore, nitrates, the final products of the oxidation of organic matter, and the chlorine introduced as common salt into all water which has been in contact with the wastes of human life, furnish additional information as to the antecedents of a sample. The results of the chlorine determination are indeed perhaps more clear than those of any other sanitary process, for chlorine and sewage pollution vary together, due allowance being made for the proximity of the sea and other geological and meteorological factors. Unfortunately, it is only past history and not present conditions which these latter tests reveal, for in a ground water completely purified, from a sanitary standpoint, such soluble constituents remain, of course, unchanged. Thus, in the last resort, it is upon the presence and amount of decomposing organic matter in the water studied that the opinion of the chemist must be based.

3. *The Quantitative Bacteriological Examination of Water.*—The decomposition of organic matter may be measured either by the material

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decomposed or by the number of organisms engaged in carrying out the process of decomposition. The latter method has the advantage of far greater delicacy, since the bacteria respond by enormous multiplication to a very slight increase in their food supply, and this fact, which necessitates great care in methods and great caution in the interpretation of results, has led hasty observers to condemn the quantitative bacteriological methods. The past ten years have witnessed, however, remarkable progress, and the establishment of a procedure for the making of approximately uniform media, through the efforts of G. W. Fuller, G. C. Whipple, and other prominent members of the American Public Health Association, has placed quantitative bacteriology on a reasonably firm basis.

The distinction between quantitative and qualitative bacteriological analysis is a somewhat arbitrary one. The gelatin plate is not only quantitative but also qualitative, inasmuch as it eliminates numerous bacteria for whose growth its conditions are unfavourable, and the dextrose broth tube is a quantitative as well as a qualitative test when it is applied to a series of measured portions of the water to be examined. A better classification would separate processes which indicate decomposing organic matter in general, from those other methods which aim at the separation of the organisms associated more specifically with sewage pollution. Under the first head comes only the standard gelatin plate count at 20°; and this count, as above suggested, roughly corresponds, in not too heavily polluted waters, to the free ammonia and "oxygen consumed," as revealed by chemical analysis. If low numbers of bacteria are found, the evidence is highly reassuring, for it is seldom that water could be contaminated under natural conditions without the direct addition of foreign bacteria or of organic matter which would induce a rapid multiplication of those already present. The bacteriologist, in such cases, can declare the innocence of the water with justifiable certainty. Recently an examination was made by one of us of a town supply which was derived from a filter gallery on the shore of a lake below a gathering ground occupied by a considerable population. Public confidence in this water had been shaken because the chlorine was high, about eight parts per million. It is true that the ammonias were fairly low, the free ammonia being about '03 parts and the albuminoid ammonia about '05 parts per million; but the gelatin count proved far more instructive. Plates made from water taken at different parts of the system showed from 0 to 5 colonies per cubic centimetre, indicating, in connection with sterile litmus-lactose-agar plates at 37° and negative tests for *Bacillus coli*, that at the time of examination all pollution had, without question, been effectually removed. Only repeated studies could, of course, prove this condition to be permanent, but the same objection applies to any other method of analysis.

If the gelatin count be high, the interpretation is less simple. In the first place, high numbers are at times due to the exceptional multiplication of certain water forms, which may generally be detected by inspection of the plates. Thus a sample of spring water recently received from a distance contained, on arrival, 4,200 bacteria per cubic centimetre, almost all the colonies being those of the fluorescent water bacillus. Sterile litmus-lactose-agar plates and negative tests for sewage bacteria confirmed the conclusion that in this case the high count signified only a multiplication in transit. Large numbers, under ordinary conditions, when they include a normal variety of forms, indicate, however, the presence of an excess of organic matter. Such organic matter may not mean sewage pollution; it does mean at least the presence of washings from the surface

of the ground, which always implies the possibility of pollution; and no supplies exposed to such conditions are now considered satisfactory. The close correspondence between the excess of bacteria in surface waters during the winter and spring months, and the epidemics of typhoid fever due to the same waters, brings out forcibly the reality of this danger, and emphasizes the fact that high total numbers of bacteria are always to be regarded as possibly significant.

4. *The Isolation of Intestinal Bacteria.*—The bacteriologist can, however, do more than detect the presence of general organic contamination; he can discover as well in polluted water certain semi-parasitic organisms, found in large numbers in the alimentary canal, whose presence specifically indicates the entrance of sewage. Ever since its interference with the early attempts to isolate the germ of typhoid fever from water, the *Bacillus coli* has been recognized as an organism of this character, and in recent years the researches of the English workers, A. C. Houston, E. Klein, and W. H. Horrocks, have shown the importance of the streptococci in the same connection.

The whole subject is a new one, and even in the technique of the colon test divergent opinions still exist on two points, which, it is to be hoped, may be harmonized for the sake of comparability. In the first place, the tests used in defining *Bacillus coli* are not yet quite uniform. For example, Whipple has recently¹ given forty-eight hours as the period for the nitrate, milk, and indol reactions; while twenty-four hours is the time employed in many laboratories for the two first, and seventy-two hours for the last of these tests. Whipple also includes the failure to form spores as one of the routine tests for *Bacillus coli*, which seems to us needless, as there are no forms likely to be found in water analyses which form spores while giving all the other reactions of the organism. These slight differences in method do not, however, materially affect the practical results of the colon test. The main characteristics of the bacillus, the typical reactions on agar and gelatin, and in dextrose broth, milk, nitrate solution, and peptone solution, are well understood; and the effect of the different periods used for the tests will only be manifest in the inclusion or exclusion of certain allied forms, which Whipple assumes, with some probability, to be typical colon bacilli, weakened by sojourn under unfavourable conditions. It seems to us desirable, in the present state of our knowledge, to exclude, as far as possible, these atypical forms, whose significance is still uncertain.

More serious than slight variations in the technique of the colon test is the failure to apply it quantitatively to the examination of small samples of water, cubic centimetres or fractional parts. *Bacillus coli* has frequently been found in large samples of waters of fair sanitary quality. Prescott² has shown the identity of this organism with certain lactic acid bacteria found upon grains and other cereals. Whipple remarks that, "*Bacillus coli*, if not widely distributed in nature, resembles certain common bacteria in all points covered by the usual tests;" but it by no means follows, as he states, that this throws "doubt upon the use of this organism as a conclusive test for faecal contamination." Many German bacteriologists, particularly the pupils of Kruse, at Bonn, have indeed concluded, because they could isolate colon bacilli from good waters by the examination of a series of litre samples, that the test was valueless. It is essentially, however, a question of less and more. Colon bacilli are either wanting in good waters or are so rare as not to be found in the examination of samples of one cubic centimetre; they are found in a majority of one cubic centimetre samples of water so polluted as to be dangerous for drinking;

between these two extremes their numbers vary with the amount of pollution. Klein and Houston³ in England; Jordan,⁴ and Winslow and Hunnewell⁵ in America, and very recently, Petruschky and Pusch⁶ in Germany, have exhaustively studied the distribution of the colon bacillus with uniform results. That the absence of this organism demonstrates the harmlessness of a water as far as bacteriology can prove it, no one would probably hesitate to acknowledge; and that, when present, its numbers form a trustworthy measure of its sanitary condition, the observers above quoted have proved beyond reasonable cavil.

5. *The Fermentation Test.*—In a thorough examination of a single sample of water the attempt should be made to isolate colon bacilli and streptococci, and to work them out in detail. Often, however, when a quick result is desired, or when a long series of routine observations must be made, some shorter method is desirable. The classic work of Theobald Smith early pointed out that the dextrose broth tube furnished such a rapid test,⁷ and it has since been well established that organisms found in water which at 37° produce in twenty-four hours a good quantity of gas, made up of approximately two parts of hydrogen to one part of carbon dioxide, are almost always colon bacilli. In the paper to which reference has several times been made, Whipple¹ has brought strong evidence of the value of this, which he calls the "presumptive test" for *Bacillus coli*. The diagrams there given, showing the chemical and bacteriological relation of certain surface waters grouped under six headings, according to their relative pollution as determined by inspection, are striking, and indicate that the presumptive colon test harmonizes closely with actual conditions. Whipple's scale, according to which water giving a positive presumptive test in one cubic centimetre is questionable, and in one-tenth of a cubic centimetre is probably unsafe, seems justified by all the evidence at hand.

6. *The Litmus-Lactose-Agar Plate.*—Besides the gelatin plate count at 20° and the study of dextrose broth tubes, either with determination of the gas formula alone, or with sub-cultures definitely to identify the colon bacillus and the streptococci, there is another method of examination—the direct plating of the water in litmus-lactose-agar—which has never come into such general use, but which is capable of wide and helpful application. Hazen and White,⁸ as long ago as 1891, suggested that agar plates grown at the body temperature, instead of permitting the growth of all the water species, would "present a selected number, namely, those which can live at blood heat; including, we may believe, most of the parasitic and pathogenic germs, which, under the circumstances, are the ones of greatest interest; and eliminating those species which cannot grow at the temperature of the body, and are, therefore, in most cases, as such, presumably harmless."

In 1893, Mathews,⁹ now so widely known for his physiological researches, published an account of the Wurtz litmus-lactose-agar plate, introduced into America in the previous year by Professor Sedgwick and himself, and suggested its application to the sanitary analysis of water. Fifty-nine samples of water examined gave results summarized in the overleaf table, indicating clearly what the author described as a "heat and agar differentiation."

The main object of this work was to facilitate isolation of the typhoid bacillus from water by eliminating those forms likely to mask it on the gelatin plate. It was obvious, however, that a method giving such special prominence to organisms growing at the body temperature, and to the acid formers, which have more and more come to be recognized as intestinal forms, must have a direct application to sanitary water analysis.

The general interest awakened about 1893 by the colon test perhaps accounts for the fact that the agar plate method was used less for its own

Sources of Water.	Average Number of Colonies per c.c.	
	Gelatin 20°.	Wurtz-Agar 37.5°.
Wells, springs	1,664	28
Reservoirs	153	43
Ponds	296	95
Taps	242	24
Streams	273	181

sake than as an aid in the separation of *Bacillus coli*. It has been extensively applied to this purpose by the Massachusetts State Board of Health; in 1899 the average number of colon bacilli in the Merrimac River water thus determined varied from eight per c.c. in August to 140 in December.¹⁰ Irons,¹¹ at about the same time, compared the method with the dextrose broth tube for the isolation of *Bacillus coli*, and concluded that it was less useful than the latter, especially when applied to highly polluted waters.

English sanitarians have made good use of this test, modified by the addition of one-thousandth part of phenol to the litmus-lactose-agar medium. The elaborate researches of Chick,¹² for example, showed 6,100 colon bacilli per c.c. in the Manchester Ship Canal, 55-190 in the polluted River Severn, and numbers up to 65,000 per gram in roadside mud. On the other hand, of thirty-eight unpolluted streams and rivulets, thirty-one gave no *Bacillus coli*, and the other seven gave one per cubic centimetre or less. The Liverpool tap water, snow, rain, and hail gave no colon bacilli.

Few recent authors appear to have used the 37° count and the count of acid-formers as in themselves important, although Rideal¹³ lays some stress upon the former, and mentions that a London water gave on an average only one-twelfth as many colonies on agar at 37° as on gelatin at 20°. The blood temperature count has, however, been in constant use in the biological laboratories of the Massachusetts Institute of Technology, at the University of Chicago, at Beloit College, and some other institutions, and in legal practice, with results of uniform significance.

7. *New Studies of Unpolluted Waters by means of the Litmus-Lactose-Agar Plate.*—We are strongly of the opinion that this feature of the bacteriological analysis should not be neglected, particularly in view of the fact that the litmus-lactose plate reveals the presence of the streptococci which may be overlooked in the dextrose tube. Because of the small attention paid to the method in recent text-books, and the paucity of published data as to its results, we have thought it worth while to repeat Mathew's work of 1893 by the examination of 259 samples of water. The samples covered as wide a range as possible of waters presumably not polluted by sewage, our object being to show definitely whether organisms growing at 37° and fermenting sugar are rare in such sources. Besides the public supplies of thirteen cities and towns, we examined waters from brooks, ponds, springs, deep and shallow wells, swamps, and pools in fields and woods. The samples were collected between January and May of 1903, and in some instances the spring rains had washed excessive amounts of organic matter into the waters tested. In most cases the samples were brought to the laboratory within four to six hours, and two gelatin plates, two litmus-lactose-agar plates, and three dextrose broth

EXAMINATION OF 259 SAMPLES OF WATER FROM APPARENTLY UNPOLLUTED SOURCES.

Source of Samples.	Gelatin Plates.			Litmus-Lactose-Agar Plates.						Dextrose Broth Tubes.			
	Number of plates.	Maximum number of colonies.	Minimum number of colonies.	Average number of colonies.	Number of plates.	Maximum number of colonies.	Minimum number of colonies.	Average number of colonies.	Plates showing red no. on top.	Number of tubes.	Number of tubes with gas.	Number of tubes with gas 1-0.	Number of tubes with gas 2-1.
Cambridge supply (tap) ...	5	10	240	20	94	8	15	2	11	0	15	0	0
Wakefield and Stoneham supply (tap) ...	7	14	150	20	59	13	12	2	6	0	21	0	0
Lynn supply (tap) ...	6	12	20	0	16	12	6	1	3	0	18	0	0
Brookline supply (tap) ...	1	2	—	all liquefied	—	2	21	15	18	2	3	0	0
Plymouth supply (tap) ...	6	12	70	10	35	12	6	0	2	0	18	0	0
Peabody supply (tap) ...	3	6	261	30	141	5	36	1	21	0	9	2	0
Dedham supply (tap) ...	6	12	13,500	0	3,717	11	3	0	1	0	18	0	0
Newburyport supply (tap) ...	6	12	100	10	36	12	14	2	9	0	18	0	0
Salem supply (tap) ...	5	10	267	200	282	10	23	10	14	0	15	0	0
Taunton supply (tap) ...	4	7	41	10	13	8	5	0	2	0	12	0	0
Sharon well (tap) ...	3	6	845	630	788	6	74	30	46	2	9	3	3
Medford supply (tap) ...	5	10	1,250	240	524	10	16	3	8	0	15	0	0
Milton supply (tap) ...	2	4	5,000	4,200	4,700	4	0	0	0	0	6	0	0
Westerly, R. I., supply (tap) ...	1	2	—	all liquefied	—	2	13	12	12	0	3	0	0
Brooks ...	61	122	1,010	30	223	121	83	0	7	0	183	13	0
Driven wells ...	15	30	54	0	18	30	5	0	1	0	45	0	0
Springs ...	32	64	1,270	20	294	64	13	0	2	0	95	13	0
Ponds fed by brooks ...	15	30	340	100	167	30	24	0	9	0	45	1	0
Melted snow ...	1	2	—	all liquefied	—	2	5	3	4	0	3	0	0
Ponds in fields ...	22	44	760	100	365	44	95	2	31	0	66	2	0
Pools in woods ...	22	44	580	10	181	43	14	0	3	0	65	0	0
Roadside pools ...	10	20	9,000	410	811	20	10	0	4	0	30	2	0
Stream, Blue Hill Reservation ...	1	2	—	all liquefied	—	2	0	0	0	0	3	0	0
Flow from rocks ...	2	4	60	30	47	4	2	0	0	0	6	0	0
Ponds fed by springs ...	6	12	420	60	188	12	5	0	2	0	18	0	0
Drainage from manured pasture ...	1	2	1,270	1,200	1,235	2	28	26	27	0	3	0	0
Swamps ...	3	6	310	240	269	6	10	0	6	0	9	5	0
Rain water, after twelve hours' heavy fall ...	7	14	—	all liquefied	—	14	6	0	2	0	21	0	0
Shallow well in Lynn Woods ...	1	2	20	10	15	2	1	1	1	0	3	0	0
Totals ...	259	517	—	—	511	—	—	—	4	775	41	38	3

tubes were inoculated from each, one cubic centimetre portions being used, or one-tenth of a cubic centimetre portions in plating from those samples which contained an obvious excess of organic matter. The agar plates were counted after twenty-four hours at 37°, the number of red colonies being noted, and at the same time the dextrose broth tubes were examined and the gas formula determined. The gelatin plates were counted after forty-eight hours.

8. *Results of Our Studies*.—The results of the examination are shown in detail upon the accompanying table. The gelatin counts, it will be noted, ranged high in some cases: the Dedham, Medford, and Milton supplies, the Sharon well, the roadside pools, and the drainage from a pasture giving figures which would excite suspicion. The high Milton figures may probably be explained by the fact that these samples were two to three days old when examined, a condition which obtained in no other case. The Dedham average was due to very high counts on two samples, the other plates being sterile.

The total count on litmus-lactose-agar was, in general, low, the highest average being 46 for the Sharon well and 31 for the pools in the fields. The general average for the whole 259 samples was less than 8. Colonies of strong red colour were found in only two samples, one from the Sharon well and one from the Brookline supply. In the latter case, we have reason to suspect contamination after the collection of the sample and while it was out of our hands. A very faint acid-producer, giving a mere suggestion of red colour, was present in a number of the public supplies, and proved to be *Bacillus mycoides*, or a closely related form. Finally, of the dextrose broth tubes inoculated, only forty-one showed gas, and of these, only three, made from the Sharon well, gave the proper gas formula. The organism here actually proved to be *Bacillus coli*; and as the well is situated just below a stable and barnyard, we are inclined to believe it actually polluted. Thus this apparent exception only adds to the force of the general rule.

If we consider the results of this examination of the litmus-lactose-agar plate, it becomes apparent that organisms capable of growth at the body temperature are not numerous in unpolluted waters; acid-formers are practically absent.

We have made no special comparative examinations of polluted waters, because their condition is fairly well known. The Massachusetts State Board of Health results, and those obtained by Chick for *Bacillus coli* alone, are sufficiently large to give an idea of the number of acid-formers present in polluted streams; while in some recent work at the Institute, to be published shortly, Mr. S. K. Baker has found total 37° counts ranging from 9,800 to 16,900 in water from various parts of the Charles River. Two twenty-four hour examinations of Boston sewage, made at the Sanitary Research Laboratory of the Massachusetts Institute of Technology during the summer of 1903, gave an average of 3,660,000 bacteria per c.c. on gelatin at 20°, and 2,310,000 bacteria per cc. on lactose-agar at 37°, with 550,000 acid-formers. In general, we may say that the total count on litmus-lactose-agar will be likely to run into hundreds in polluted waters, sometimes equalling that obtained on gelatin at 20°, with a large proportion of acid-formers; while good waters will show a count under fifty, with acid-formers only rarely present. From exceptionally pure waters sterile plates at 37° may often be obtained.

9. *Summary of Bacteriological Methods*.—In conclusion, it appears, in the light of present knowledge, that the full bacteriological examination of the future will consist of three parts: the gelatin plate count as an

estimate of the amount of organic decomposition in process; the total count and the count of red colonies on litmus-lactose-agar, as a measure of the organisms which form acids and thrive at the body temperature; and the study of a series of dextrose broth tubes for the isolation of colon bacilli and streptococci. The simple examination of the dextrose broth tube and the count on the litmus-lactose-agar plate will serve for what Whipple has well called presumptive tests.

10. *Significance of Bacteriological Results.*—The results of the bacteriological examination have, in several respects, a peculiar and unique significance. First, this examination is the most direct hygienic method of water analysis. Whipple, in the paper quoted above, says that his presumptive test “gives results that agree well with chemical and biological analysis.” Bacteriology, however, needs no such indirect justification. What we dread in drinking water is the presence of pathogenic bacteria, mainly from the intestinal tract of man, and it is quite certain that the related non-pathogenic bacteria from the same source will reveal the presence of these disease germs more clearly than will any chemical compounds. In the second place, the bacteriological methods are far more delicate than any others. Klein showed by experiment with dilutions of sewage that the colon test was from ten to one hundred times as sensitive as the methods of chemical analysis; and a simple calculation shows that this must be the case, since sewage contains only from a hundred to a thousand times as much chlorine as good drinking water, against ten thousand to a hundred thousand times as many colon bacilli. Studies of the self-purification of streams have confirmed these conclusions on a practical scale. Thus in the Sudbury River it was found that while the chemical evidences of pollution persisted for six miles beyond the point of entrance, the bacteria introduced could be detected for four miles further.¹⁴ Thirdly, negative tests for *Bacillus coli* and low bacterial counts may be interpreted as proofs of the good quality of water, with a certainty not attainable by any other method of analysis. Many a surface water with reasonably low chlorine and ammonia has caused epidemics of typhoid fever; but it is impossible, under any natural conditions, that a water could contain important typhoid contamination without giving clear evidence of pollution in the dextrose broth tube or on the lactose-agar plate.

It seems to the writers that the real application of chemistry begins where that of bacteriology ends. When pollution is so gross that its existence is obvious and only its amount needs to be determined, the bacteriological tests will not serve on account of their excessive delicacy. In studying the heavy pollution of small streams, the treatment of trades wastes, and the purification of sewage, the relations of nitrogenous compounds and of oxygen compounds are of prime importance. In other words, when pollution is to be avoided because the decomposition of chemical substances causes a nuisance, it must be studied by chemical methods. When the danger is of infection, and comes only from the presence of bacteria, bacteriological methods furnish the best index of pollution.

In the study of certain special problems the paramount importance of bacteriology is generally recognized. The distribution of sewage in large bodies of water into which it has been discharged may be traced best in this way, on account of the ready response of the bacterial counts to slight proportions of sewage, particularly since the ease and rapidity with which the technique of plating can be carried out make it possible to examine a large series of samples with a minimum of time and trouble. For the same reasons, and because the factors which condition the disappearance of

disease germs from a polluted river must operate most nearly in the same manner upon related parasitic forms, the fermentation tube and the gelatin and lactose-agar plates furnish the best criteria for a study of the self-purification of streams. Finally, the quickness with which slight changes in the character of a water are marked by fluctuations in bacterial numbers, renders the bacteriological methods invaluable for the daily supervision of surface supplies or of the effluents from municipal filtration plants.

In the commoner case, when normal values obtained by such routine analyses are not at hand, the problem of the interpretation of any sanitary analysis is a more difficult one. The conditions which surround a source of water supply may be constantly changing. No engineer can measure the flow of a stream in July, and deduce the amount of water which will pass in February. Yet the July gauging has its own value and significance. So a single analysis of any sort is not sufficient for all past and future time. If it gives a correct picture of the hygienic condition of the water at the moment of examination, it has fulfilled its task; and this the bacteriological analysis can do. The evidence furnished by inspection and by chemical analysis must be welcomed whenever it can be obtained. Yet we are of the opinion that, on account of their directness, their delicacy, and their certainty, the bacteriological methods should be the last to be omitted, and, if necessary, they alone may furnish conclusive testimony as to the safety of a potable water.

In conclusion, we desire to express our thanks to Professor W. T. Sedgwick, whose interest in this subject has been our chief inspiration, as it was that of some of its earliest investigators in 1891 and 1893.

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