ON THE VALUE OF TESTS FOR BACTERIA OF SPECIFIC TYPES AS AN INDEX OF POLLUTION.*

BY H. W. CLARK AND STEPHEN DeM. GAGE, LAWRENCE, MASS.

In recent years increased attention has been given, by those interested in sanitary problems, and especially water supply and water purification, to methods for the determination of fecal bacteria in water, and to the significance of their presence as a determining factor in judging the degree of purity of a water.

During the past eight years the work at the Lawrence experiment station along this line has covered a wide field, and over 18,000 samples, including 4,700 samples of polluted river water, 9,100 samples of filtered waters, 2,200 samples of other potable waters, from springs, curb wells, tubular wells, domestic wells, ponds and other surface supplies, etc., and 2,100 miscellaneous samples, including shell-fish, seawater, ice, milk, dust, excrement from men and animals, grains, food stuffs, etc., have been examined. Investigations have been made upon the seasonal distribution of B. coli, upon the elimination of B. coli from water by storage and by freezing, upon the relative occurrence of the various types of fecal bacteria in different classes of samples, upon the value of the various methods for the determination of fecal bacteria, and upon the relative viability of B. coli and B. typhosus under a variety of conditions.

SIGNIFICANCE OF THE VARIOUS TEST TYPES.

Of the various forms of bacteria used as indices of sewage pollution, the principal ones are B. coli, the sewage streptococcus and B. sporogenes. Of these, B. coli has formed a basis for the greatest amount of work; nevertheless, the other two have considerable standing among various observers in different parts of the world. B. coli is supposed to show present or past pollution, usually recent pollution. The streptococcus type, it is stated, should show only very recent pollution by sewage; evidence as to this, however, has not been forthcoming. The bacillus sporogenes, being a spore-forming organism, may show present pollution and it may also show pollution which has occurred at some previous time, and from which the danger has now entirely ceased. The two so-called paracolon types, that is, the aerogenes, or enteridi-

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tis, Gärtnner, type and the chologenes type, are introduced here merely because in some laboratories one or the other is included under the colon type. As to the distribution of the colon, aerogenes and chologenes types, there is a large amount of data. The data concerning the streptococcus type is not as complete. Houston describes twenty-one cultures, among which there appear to be at least seven distinct species, while the same number of species have been isolated and described at the Lawrence experiment station. Winslow and Hunnewell report the finding of sewage streptococcus in a variety of apparently unpolluted samples. It certainly is a fact that micrococcus forms in general are abundant in the air and in the soil. The data concerning B. sporogenes, belonging as this organism does to that restricted and little studied group of bacteria, the pathogenic, spore-forming, obligate anaerobes, is very meagre indeed. At present we know of only two other species which would be included by the usual sporogenes test.

In the examination of water or other substances for present pollution, tests for the specific organism which is most numerous in sewage and for which the tests are most exact, are of the most value, always assuming that this organism is characteristic of sewage and is not found, or at least is only occasionally found, in sources removed from pollution. Examinations of a large number of samples of sewage at the experiment station show that in dilutions \(1 : 10,000\) we may expect to find B. coli in 95 per cent. of the samples, and that we may identify the sewage streptococcus in 67 per cent. of the samples. A dilution of \(1 : 50,000\) cuts the percentage of positive tests for B. coli to 78 per cent. of the samples, and the percentage of streptococcus to 6 per cent. of the samples. In dilutions of \(1 : 100,000\) B. coli was found in 40 per cent. of the samples, and in a dilution of \(1 : 1000,000\) B. coli was found in 6 per cent. of the samples. The sewage streptococcus could not be identified in dilutions greater than \(1 : 50,000\). B. sporogenes can usually be detected in dilutions of \(1 : 500\) to \(1 : 1,000\), but in dilutions greater than this it is rarely detected. In other words, we may expect to obtain a diagnosis with B. coli in such dilutions that the presence of B. sporogenes or of the sewage streptococcus could not be detected.

During a period of six months in 1902 and 1903, daily examinations were made for these three organisms in the Merrimack river water and in the effluent from the Lawrence city filter. In the polluted river water, when testing one cubic centimeter, B. coli were found in 90 per cent. of the samples examined, bacteria of the sewage streptococcus type were found in 45 per cent. of the samples, and B. sporogenes were found in only 24 per cent. of the samples. In the filtered water,
B. coli were found in 5 per cent. of the one cubic centimeter samples, and in 10 per cent. of the 100 cubic centimeter samples. The sewage streptococcus was not found in volumes as large as 100 cubic centimeters. B. sporogenes was not found in one cubic centimeter but was found in 14 per cent. of the samples when testing 100 cubic centimeters.

During a period of four months in 1903, samples of milk from Lawrence dealers were tested daily for fecal bacteria. B. coli were found in 4 per cent. of these samples, bacteria of the sewage streptococcus type were found in 3 per cent. of the samples, and B. sporogenes were found in 10 per cent. of the samples.

A number of investigators have published results seeming to show the presence of bacteria of the colon type in samples from sources apparently removed from pollution, and these results tend to discredit the value of tests for organisms of this type as an indication of sewage pollution. That B. coli are widely distributed is not to be disputed when we consider that this organism is found in the fecal discharges of men and the major animals widely used as a dressing for arable soil, and in the intestinal contents of many of the smaller animals, both domestic and wild, and in the evacuations from fowls and some of the migratory birds. Flies and other insects may also act as carriers of these germs, depositing them in places where we would least expect to find them. Examinations of 48 flies at the experiment station resulted in finding B. coli in or on nine of these, and bacteria of the sewage streptococcus type in or on three others. No relation could be traced between the locality where these flies were caught and the finding of the test organisms.

Nevertheless, corroborated evidence that the finding of organisms of the colon type in sources apparently remote from pollution is proof that these bacteria are natural rather than chance inhabitants of these sources, has not been forthcoming, such findings, on the other hand being additional evidence of minute pollution and of the extreme delicacy of the test by which we are able to detect it.

The fact that Klein and Houston\(^4\) found organisms similar to B. coli in commercial food stuffs, and have been supported in their findings by a number of other investigators, has been the basis of an attempt to show that the colon bacillus is merely a member of a larger group of saphrophytes of the so-called lactic acid group. These critics, however, fail to note the fact that Klein and Houston also found in these same samples of cereals, bacteria of the sewage streptococcus type and of the B. sporogenes type, both of which types are offered as indicating sewage pollution. If we accept the fact that these observers found B. coli, we must also acknowledge that, as shown by other bacterial tests made by them, the grains which they tested had at some
time been exposed to pollution. At the experiment station, a number of samples of grains have been examined for B. coli, all of these samples, however, being carefully collected directly from the fields where they were growing, and in no case has any organism been found which would be confounded with the colon type, notwithstanding the fact that 25 samples of corn were examined which were grown upon a municipal sewage filtration area to which sewage was being applied daily.

THE SO-CALLED PRESUMPTIVE TEST AND ITS SIGNIFICANCE.

In some laboratories work upon the determination of fecal bacteria of the colon type is limited to what is known as the presumptive test, that is, the formation of gas in dextrose broth inoculated with a sample of the water under examination. In other laboratories the procedure corresponds with that used at Lawrence,* except that the test for indol or that for the reduction of nitrates is omitted.

A study of the various compilations of bacterial description shows that there are at least 58 well-described species of bacteria of five primary types which would give positive results; that is, be designated as colon bacilli, if we depended upon a presumptive test alone.

Of these 58 species, 12 would be included in the colon type, as it is interpreted at Lawrence, three species are of the chologenes type (bacteria identical with those in the colon type, with the exception that nitrous acid is not formed in nitrate pepton solution), seven species belong to the aerogenes, or enteriditis, Gartner, group (bacteria identical with the colon type with the exception that indol is not produced in pepton solutions and thirteen species would give positive reactions by the presumptive test, in which the descriptions are not sufficiently worked out to identify them with or exclude them from the three preceding groups.

Besides these there are 23 species which would show positive results when tested by the presumptive test alone, which would be readily thrown out by subsequent tests and would never be included in either of the first three groups.

In studies previously reported it was shown that the presumptive test had an efficiency of from 40 to 60 per cent. as an indication of the presence of B. coli, this factor varying with the character of the sample and the degree of pollution. The presumptive test in itself, without confirmatory tests, would, even to the experienced observer, be very difficult of correct interpretation. In the examination of surface water supplies, the presumptive test in small volumes would appear to be of

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little value. As the volume was increased, however, to 100 cubic centimeters the presumptive test gave results which agreed more closely with the probable pollution, and with liter samples this connection between the probable pollution and the presumptive test was even closer.

In the examination of well waters of different types the presumptive test frequently gave positive results where the absence of B. coli, and the general character of the samples from all standpoints, showed the samples to be entirely above suspicion. In the examination of shellfish the presumptive test unconfirmed would lead to very erroneous results. With the shell-fish from sources known to be grievously polluted a positive result was obtained with the presumptive test in only about 80 per cent. of the samples. With samples which were known to be remote from pollution the results were even more misleading, 25 per cent. of the examinations of the intestines of shell-fish from such sources, and 3 per cent. of the samples of sea-water from those sources, giving a positive presumptive test, although B. coli or other fecal bacteria was not found. In the examination of milk over 14 per cent. of the samples showed a positive presumptive test, but something less than 4 per cent. of the samples showed fecal bacteria when the tests were concluded.

In the examination of ice, 5 per cent. of the samples gave a positive presumptive test in one cubic centimeter, as compared with 3 per cent. positive in 100 cubic centimeters. The findings of B. coli, however, were 1 per cent. in one cubic centimeter and 2 per cent. in 100 cubic centimeters, probably nearer the true relation.

**B. coli in Filtered Water.**

For several years the B. coli work at Lawrence was almost entirely confined to determinations of this organism in the Merrimack river water and in the effluents of the filters receiving this water. In these studies many thousand tests were made and the work of the Lawrence city filter and of the various experimental filters, operated under a variety of conditions have been observed. This work has already been summarized in the reports of the Massachusetts Board of Health since 1896, in the chapters upon “Filtration of Water.”

In the filtration of polluted water, such as that of the Merrimack river, bacterial tests are, as well known, of more value than chemical analyses. The filtered water may be changed but little from the raw water by filtration as far as chemical analyses can determine, and yet a source of danger be absolutely removed as shown by bacterial tests. Long continued comparisons of the bacterial examinations, as applied to raw and filtered waters, have shown that the colon test is a more
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delicate indication of filter efficiency, when filtering water as polluted as that of the Merrimack river, than tests of the numbers of bacteria in the filtered and unfiltered waters. Disturbing factors in filtration, shown slightly or not at all by ordinary bacterial counts and comparisons, and yet serious enough to affect the health of a city using filtered water are shown strongly by B. coli tests.

B. COLI IN SPRING WATER.

During an investigation of spring waters upon the market in Massachusetts, made by the State Board of Health in 1900, an account of which was published in the report for that year, 282 samples from ninety-six sources were examined. Fourteen of these samples, or less than 5 per cent., were found to contain B. coli, these fourteen samples being from thirteen different sources. Of the 14 samples in which the germ was found, 6 were collected from springs by employes of the Board, the remaining 8 being samples taken from original packages of the water as supplied by the spring water people to their customers, and the B. coli present in these last samples could probably be traced to carelessness and uncleanliness in bottling or handling the waters.

Of the 6 samples collected directly from the springs, in which B. coli was found, one was from a source which inspection and chemical analysis showed to be unpolluted; one was from a source surrounded by cultivated fields, but without buildings in the immediate vicinity (the chlorine in this sample was above the normal for the region); the other four samples were from locations with either buildings, barnyards, vaults, etc., in the immediate vicinity, and in which the chlorine was above the normal in each case; in other words, in an examination of 282 samples of spring waters, B. coli was found in less than 5 per cent. of the samples, and in most of the samples collected by employes of the Board, in which it was present, evidence of probable pollution was shown by inspection of the surroundings as well as by chemical analysis.

B. COLI IN WELL WATER.

During the past two or three years 170 samples from twenty-five sets of tubular wells and nineteen curb wells, used as sources of public water supply, have been examined for B. coli. Of the 128 samples from tubular wells which have been examined, only one has given a positive test for B. coli, and that when testing 100 cubic centimeters of the water. In the one instance where a positive test was obtained in a sample from a tubular well, this water had been shown to be suspicious by many chemical analyses, and these wells are near one of the most polluted rivers in the State.
Besides the samples from tubular wells, already enumerated, positive tests were given for B. coli in several samples of water from a supply system drawn from tubular wells, this water having become accidentally contaminated by the introduction of polluted surface water at this time sufficient to cause an outbreak of typhoid fever among the consumers of the water. Chemical analysis and determination of numbers of bacteria failing, however, to detect this pollution.

Of the 42 samples from curb wells, one has given a positive test for B. coli when testing one cubic centimeter, and 3 when testing 100 cubic centimeters. The waters in these wells are, however, as shown by chemical analysis and the health of the people using them, probably of a good and safe quality. In the curb wells, many feet in diameter, there is, perhaps, a chance for accidental contamination by B. coli to which the tubular or driven wells are not subject.

Of five hundred and fifteen samples of water from one hundred and eighty-six private or domestic wells, examined during the past two years, 14, or 2.7 per cent., have given a positive test for B. coli when testing one cubic centimeter, and 17, or 3.3 per cent. when testing 100 cubic centimeters of the water.

Studying the complete chemical and bacterial analyses of all these samples of shallow well water, it is evident that gross previous or present pollution of the water, as indicated by high chlorine, unoxidized nitrogen in the form of free ammonia, and oxidized nitrogen or partially oxidized nitrogen in the form of nitrates and nitrites, and positive tests for B. coli in conjunction, was the exception rather than the rule. That is to say, waters that might at some time become dangerous to those using them were more clearly indicated by single chemical analyses than by one or two B. coli tests, or by determinations of the number of bacteria present.

The presence of B. coli at the time of examination may indicate actual danger to health and its absence even in the most polluted of these waters, chemically, may indicate lack of imminent danger, but the chemical analyses are certainly the more decisive.

B. COLI IN SURFACE WATERS.

During the past three years 432 samples from ponds and lakes, within twenty miles of the experiment station, have been examined bacteriologically, tests for B. coli having been made in one and one hundred cubic centimeters during 1901 and 1902, and in one, one hundred and one thousand cubic centimeters during 1903.

Of the 30 samples collected from the lake with the greatest population per square mile of watershed, 5, or 13.3 per cent. of the number collected, gave positive tests when taken one cubic centimeter.
Lake No. 2, with the next greatest population per square mile of watershed, gave positive tests in 3.5 per cent. of the one cubic centimeter samples examined.

Of the two remaining lakes, samples from which gave positive tests for B. coli in one cubic centimeter of water, both have a comparatively small population per square mile of watershed, but both are used more extensively than any other lakes (except perhaps No. 1) for boating and fishing, and both are surrounded by cultivated land and have picnic grounds upon their shores.

Studying the results of tests for B. coli in these waters when testing larger volumes than one cubic centimeter, it was found, that with one exception, even those lake waters not responding positively to this test in one cubic centimeter, gave occasional positive tests in 100 cubic centimeters, and that when one liter was examined, the percentage of positive tests again increased in every instance but one. The results show with considerable clearness that in the examination of surface waters from inhabited localities, the question of obtaining positive tests for B. coli is only one of taking a sufficient volume of water for the test. They also indicate that the degree of contamination of these waters varies inversely as the volume required to give a positive test for B. coli.

EXAMINATION OF SHELLFISH.

During the past three years many samples of shellfish such as clams, oysters, quahogs, scallops, etc., and samples of sea water from the areas whence these shellfish were gathered have been examined at the experiment station for evidence of pollution. In the shellfish samples tests were made for B. coli and for the sewage streptococcus in the body of the shellfish itself and in the juice or water in the shell.

Enough study has been made by many investigators to show that B. coli is not a normal inhabitant of the intestines of clams or oysters, and that its presence in the intestines or juice in the shell, must be due to contamination either by drainage and sewage flowing over the clam and oyster beds, or by careless and uncleanly handling of the shellfish between the time of collecting and placing upon the market. In this work, therefore, the ability to demonstrate clearly the presence of a specific sewage organism such as B. coli, is an invaluable aid in determining the question of purity or pollution.

The following table shows the number of samples of shellfish examined, together with a more or less accurate statement as to the character of the source from which these fish were taken:
**TABLE SHOWING RESULTS OF TESTS FOR B. COLI IN SHELLFISH AND SEA WATER FROM POLLUTED AND NON-POLLUTED SOURCES.**

<table>
<thead>
<tr>
<th>Character of source</th>
<th>Number of sources</th>
<th>Number of samples</th>
<th>Per cent. of samples positive</th>
<th>Shellfish</th>
<th>Sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shell water</td>
<td>Intestine</td>
</tr>
<tr>
<td>Not polluted</td>
<td>15</td>
<td>10</td>
<td>44</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Doubtful</td>
<td>22</td>
<td>19</td>
<td>114</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>Polluted</td>
<td>6</td>
<td>4</td>
<td>49</td>
<td>48</td>
<td>75</td>
</tr>
</tbody>
</table>

In order to acquire more definite information as to the accuracy of the methods for detecting B. coli in shellfish, an extended investigation was made of one clam bed at the mouth of a river polluted by the sewage of a number of large cities, and at one end of the bed, emptying into the river was one of the principal sewers of a city with a population of about 15,000, the clam flats extending from the sewer outfall about one and three-fourths miles to the mouth of the river. In this investigation it was noticed that of the samples taken at varying distances from the sewer outlet, the greatest percentage of positive tests for B. coli was found in samples collected at a distance of one-half to three-fourths of a mile from the sewer. Samples collected at a distance of one-fourth to one half of a mile also gave a greater percentage of positive tests than did samples collected nearer the sewer outlet.

The surface of this clam bed near the sewer and extending perhaps one-eighth of a mile away was covered with a layer of slime (sewage sludge) in an active state of putrefaction, and, as is well known, the organisms of putrefaction are inimical to bacteria of the colon type.

**RELATIVE VIABILITY OF B. COLI AND B. TYPHOSUS.**

In the use of tests for B. coli in tracing the source of epidemics of typhoid fever, a knowledge of the relative viability of B. coli and B.
Typhosus is of considerable importance. Extensive studies along this line at the experiment station have shown us that there is a great similarity between the length of life of these two germs under a variety of conditions. A brief resume of these experiments is given in the following table:

**Table Showing Relative Viability of B. Typhosus and B. Coli Under Certain Conditions.**

<table>
<thead>
<tr>
<th>Removal by water filters.</th>
<th>B. typhosus</th>
<th>B. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99-100 per cent. removed.</td>
<td>99-100 per cent. removed.</td>
</tr>
<tr>
<td>Persistance in filtering material after infection ceased.</td>
<td>24-48 hours.</td>
<td>24-48 hours.</td>
</tr>
<tr>
<td>Effect of temperature just before freezing on fluid cultures.</td>
<td>98-99 per cent. killed in 24 hours. May live 5-15 days.</td>
<td>98-99 per cent. killed in 24 hours. May live 12-21 days.</td>
</tr>
<tr>
<td></td>
<td>97 per cent. killed in one hour. 99 per cent. killed in 24 hours. May live 280 days.</td>
<td>99 per cent. killed in one hour. 99 per cent. killed in 24 hours. May live 135 days.</td>
</tr>
<tr>
<td>Effect of freezing.</td>
<td>95-99 per cent. killed in 15 minutes. Usually sterilized in 3-6 hours.</td>
<td>80-99 per cent. killed in 15 minutes. Usually sterilized in 2-4 hours.</td>
</tr>
<tr>
<td>Resistance to sunlight when spread out in thin layer.</td>
<td>99 per cent. killed at 80°C. Few individuals may survive up to 80°C. Sterilized at 80°C.</td>
<td>99 per cent. killed at 50°C. Few individuals may survive up to 75°C. Sterilized at 80°C.</td>
</tr>
</tbody>
</table>

**Conclusions.**

The principal types of bacteria used as indices of pollution are the colon type, the sporogenes type, the sewage streptococcus type, and occasionally the aerogenes and the chologenes types. The colon type has received the most study, and since it is the most numerous of these types in normal sewage, it is of the most value as a positive indication of sewage pollution.

Results of examinations for B. coli in different volumes of water show the tests in both one and 100 cubic centimeters are of value, the tests in the larger volumes being confirmatory of the tests in the smaller volume. In cases where a considerable number of samples are taken from the same source, the tests in the larger volume frequently give more information as to the quality of the water than do the tests in one cubic centimeter.
As an indication of the presence of B. coli the presumptive test, so-called, has, judging from our own work, little value unless supported by subsequent tests.

In the filtration of a polluted water, such as that of the Merrimack river, bacterial tests are of more value than chemical analyses. The filtered water may be changed but little from the raw water by filtration, as far as chemical analyses can determine, and yet a source of danger may be absolutely removed, as shown or strongly indicated by bacterial tests. Disturbing factors in filtration shown slightly or not at all by ordinary bacterial counts and comparisons are often shown strongly by B. coli tests.

In the examination of ground waters from springs, tubular wells and curb wells, the results of chemical analyses and B. coli tests were generally in accordance in indicating purity or pollution. In the examination of samples of spring waters collected in a proper manner from carefully guarded springs, the degree of the purity of the samples is shown almost absolutely by chemical analyses. Tests for B. coli may, however, show pollution due to carelessness at the spring or in handling the bottled waters which cannot be detected by chemical means.

The complete analyses of samples from a large number of domestic wells show that polluted waters that might become unfit for consumption at any moment are more plainly indicated by a single chemical analysis than by a single determination of B. coli. The presence of B. coli at the time of examination may indicate actual danger of health and its absence even in the most polluted of these waters — chemically — may indicate lack of imminent danger, but the chemical analyses are certainly the more decisive.

The results of the examinations of water from ponds and lakes show with considerable clearness that in the examination of surface waters from inhabited localities, the degree of contamination of these waters varies inversely as the volume required to give a positive test for B. coli, and that the question of obtaining positive tests for B. coli is only one of taking a sufficient volume of water for the test.

In the examination of shellfish from suspected sources, the determination of the fecal bacteria is of considerable importance, as showing the purity or pollution of these sources.

Outbreaks of typhoid fever coincident with the finding of B. coli in normally pure waters, i. e., in water known to be safe under ordinary conditions but becoming polluted by the entrance of polluted water, have occurred within the observation of the writers, where the danger was not indicated by chemical tests or by a significant increase in the numbers of bacteria beyond the usual limits of variation for waters of their class.
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Comparative tests of the relative viability of B. typhosus and B. coli show that there is a very great similarity between the length of life of the two germs under a variety of conditions, and that under some circumstances the positive test for B. coli may be interpreted to indicate danger from the typhoid germ.

REFERENCES.
5. Gage, thirty-third annual report Massachusetts State Board of Health, 1901, page 397.