Bacteriological survey of "surface" water supplies.

BACTERIOLOGICAL SURVEY OF "SURFACE" WATER SUPPLIES.

THERE are two contending interests which the chemist and the bacteriologist have to face when they have to give an opinion on any water supply.

Firstly, they have to protect the consumer against any possible danger that might arise from the use of bad water.

Secondly, they have to consider the ratepayer, that is to say, they must not cause to the community any unnecessary trouble or expense by condemning, without good reasons, any source of water supply.

It is my object in this communication to show how a bacteriological investigation may be carried out so as to meet the difficulties of the case and to protect all interests concerned.

I have often been struck with the *absence of any sure guide to the interpretation of the results of bacteriological analysis of water.* This difficulty has apparently been felt by most of the bacteriologists who have paid any attention to this question. Most writers allude to it without giving any solution, apparently reserving to themselves the liberty to use their own judgment in the interpretation of their results. Others have, on the contrary, laid down definite rules. Unfortunately, among the latter few will be found to agree with each other.

Thresh (1896) rightly says that "the attempt to set up a standard of purity based on the number of micro-organisms in a given quantity is as illogical as the old chemical standards."

Having for several years acted on this belief, I am in perfect agreement with him. I cannot, however, altogether follow the same authority when he states: "At the present time it is doubtful whether a biological examination really tells us more than a chemical analysis, and very often it cannot tell us as much."

During the last five years I have investigated bacteriologically the water supplies of some important towns, and have had the advantage of being able to compare my results with those of my colleague, Professor H. Dixon, who examined chemically samples of nearly all the waters which were examined bacteriologically by me. I am quite satisfied that both the chemical and the bacteriological examinations were useful.

The bacteriological examinations, however, revealed slight pollutions much more surely and clearly than the chemical examination.

If of the two methods one had to be chosen, I am absolutely certain that the bacteriological method would be the more useful; but a thorough investigation of any water supply should always include a chemical as well as the bacteriological analysis.

My remarks here will, however, be chiefly confined to the latter method.

Our present position with regard to the value and interpretation of bacteriological results will be made clear by a few references to the views held by several authorities.

Koch (1885) says that the number of micro-organisms in water is of the greatest importance, as it indicates whether or not the water is contaminated with organic matter undergoing decomposition. When decomposing organic matter, which always contains a large number of bacteria, gets mixed with water, this water becomes rich in micro-organisms. Even if one were unable to discover any pathogenic germs in such a contaminated water, the fact that it contains decomposing organic products, among which pathogenic bacteria might be present, is enough to render this water suspicious.

In his well-known paper on water filtration, Koch, in 1893, has fixed at 100 the maximum number of colonies that may be allowed to be present in water properly filtered through sand. Koch admits at the same time that a few of the bacteria which are found in the unfiltered water may pass through the filter and be found in the filtered water. There does not seem to me, therefore, any very good reason for admitting a standard for unfiltered and another standard for filtered water.

Supposing we admit a numerical standard, we must, if we follow Koch, regard 100 bacteria as the highest number compatible with purity of drinking water.

Miquel (1891) has given a scale of purity, which I give only to show the arbitrary nature of the classification of waters based on numbers only. It must be remembered that the methods used by Miquel reveal a larger number of bacteria than the usual methods:—

Excessively pure water	...	0 to	10 per 1	cubic centimetre.
Very pure ,,	...	10 to	100 ,,	,,
Pure ,,	...	100 to	1,000 ,,	,,
Mediocre (or passable) water	...	1,000 to	10,000 ,,	,,
Impure water	...	10,000 to	100,000 ,,	,,
Very impure water ...	...	100,000 and over	,,	,,

Crookshank gives in 1896 the following scale, equally arbitrary:—

Very pure water may contain up to	100 bacteria to the cubic centimetre.
Water containing	1,000 bacteria, or more, should be filtered.
Water containing more than	... 100,000 bacteria is contaminated with surface water or sewage.

Macé (1897), after explaining that the mere number of bacteria must be taken only as an indication and not as affording an absolute criterion, gives the following scale of purity :—

Very pure water	0 to	10 bacteria	to the cubic centimetre.
Very good ,, ...	20 to	100 ,,	,,
Good ,, ...	100 to	200 ,,	,,
Passable (mediocre) water...	200 to	500 ,,	,,
Bad water	500 to	1,000 ,,	,,
Very bad water	1,000 to	10,000 and over ,,	,,

Migula (1890) argues that the mere number of the colonies affords us no means of judging of the fitness of water for drinking purposes, but that, on the other hand, a great deal depends on the number of kinds present.

Good pure spring water from mountains contains only a few species ; water which has been contaminated by drainage contains, on the contrary, an exceedingly great number of species.

Migula holds that there should never be more than ten different species of bacteria in good drinking water. He qualifies this statement by saying that a water containing fewer species may have to be condemned on account of the nature of the bacteria, whilst sometimes a water containing more than ten kinds may be considered fit for drinking purposes.

As regards the number of colonies, Migula is inclined to admit a maximum limit of 500, *i.e.*, the limit admitted by most observers. For a time bacteriologists attached a considerable importance to the presence or absence of liquefying bacteria, but I think that as there are several rapidly liquefying bacteria often present, even in unpolluted water, it is necessary to distinguish between those liquefying bacteria which are associated with pollution and those which are not, if the presence of liquefying bacteria is to be used as a criterion at all.

Meade-Bolton, Lustig, G. Roux, all well known in connection with the bacteriological examination of water, have expressed views similar to but less categorical than those just quoted.

I need not say more to show that there is, as yet, no consensus of opinion among bacteriologists with regard to the interpretation of the results of water analysis. There is, however, a general tendency to admit that much judgment has to be used in interpreting these results.

There is no difficulty with regard to very bad waters. Those who propound numerical standards all agree that a water containing 1,000 germs is not good. This in itself is already a very important point gained, for in many suspicious or bad waters which might chemically appear good, the presence of organic impurities can easily be detected in this way. But when we have to deal with waters containing less than 1,000 bacteria to the cubic centimetre, there is a considerable divergence in the views expressed by various writers.

In presence of such conflicting evidence it would seem impossible to give any definite opinion, except on very bad waters, or on waters containing some definite pathogenic germ capable of isolation.

This would considerably restrict the use of the bacteriological examination of water.

Under the influence of these considerations I determined several years ago to adopt a *comparative method* in all my investigations. This method has yielded me so far results which appear free from ambiguity when applied to the investigations of surface and subsoil water, such as may be found in open streams, land drains, catchwaters, impounding reservoirs, filters, service reservoirs, and mains.

As there is *no general standard of purity* that can be relied upon, it seemed to me evident that in almost any upland gathering-ground one should be able to find a few uncontaminated feeders, and that bacteriological examination of the water of *these uncontaminated feeders would give the standard wanted.*

To find such feeders the bacteriologist must of course inspect the gathering-grounds himself, and after noting the configuration and nature of the ground, the course of the feeder, its relation to the slopes which it drains, the absence or presence of cultivated areas, of paths, of houses, the possibilities of human traffic, the presence of cattle or sheep, he can then determine whether the feeder inspected is likely or not to be contaminated.

In upland gathering-grounds there are areas situated near the upper boundary of the watershed, and entirely uncultivated. Some of these areas are rocky, barren, or at best rough pastures, which can only be used as sheep-runs. Cattle have either no access to them or pass through them unfrequently. They are away from human traffic and above any habitation.

Rain-water, falling on such areas, may run on the surface, when the soil is not retentive, and then form brooklets or streams, or else it may partly penetrate into the upper strata of the soil, and then passing into natural underground channels, or superficial land drains, it emerges from the sides of the slopes in the form of springs, and ultimately joins natural streams or some artificial catchwater leading it to the impounding reservoir.

Water collected from these brooklets, natural springs, or as it emerges from field drains, in these uninhabited, unfrequented, and uncultivated regions, above any inhabited house, may be considered as representing the purest surface water that can be collected under the conditions peculiar to the gathering-ground under investigation.

It is necessarily free from any excess of bacteria associated with decomposing organic matter, human or animal diseases (provided no carcass of a dead animal is found in its neighbourhood). Such a water

should be good, provided no abnormal chemical constituents were present.

Even under these conditions water is liable to variations, according to the state of the weather; during heavy rains it becomes mixed with surface soil and some decayed vegetable matter washed down from the surrounding slopes or carried away from the banks of the brook itself. Consequently, during and after stormy weather, even the water of a pure feeder contains a much larger number of bacteria than it would contain when the weather is fine and the stream is running smoothly. This increase of bacteria, which is not excessive when the surface soil is of suitable nature, does not render the water dangerous to health. The rain can only take into the stream the washings of uncontaminated surfaces comparatively free from decaying animal or vegetable matter. Knowing the number and the kinds of bacteria normally present in two or three uncontaminated feeders both during fine and rainy weather, one would therefore have standards by which to test the water collected on any other parts of the gathering-ground, or taken from the reservoirs or mains within the limits of the same supply.

If we suppose that, descending from the higher grounds to the lower levels, one finds, at some distance from the banks of a feeder, a farm, a manured field, or anything else that might be a source of contamination, it will be enough to take a sample of water at a place where the washings or drainage from the suspected area may reach the feeder (after ascertaining that the nature of the banks and bed of the stream have not altered). By comparing this water with the water of the same feeder taken above the suspected place, it will be easy to ascertain whether important changes have taken place.

To make the comparison absolutely reliable, the two specimens should be taken at as short an interval of time as possible, say a quarter of to half an hour.

If any sewage has passed into the stream, a considerable increase in the number of bacteria will be found, and not only will the actual number of bacteria be increased, but also the number of kinds.

The specimens of water having been collected under the same conditions and within a short interval of time, the increase in the number of bacteria cannot be due to any multiplication having taken place in the water. The banks and the bed of the stream not having altered in character, the increase of bacteria must be taken to indicate the addition of decomposing organic matter to the water of the stream. It may be that the possible source of pollution is sufficiently removed from the stream to have little or no effect upon it when the weather is fine, and the decomposing matter can be dealt with by the nitrifying bacteria of the soil. Therefore, before concluding that the possible source of pollution is innocuous, it will be necessary to examine again

the stream during rainy weather. When the soil is soaked with water, and surface water rushes rapidly down the slopes into the streams, a considerable amount of pollution may take place which would not be evident during fine weather. Hence the necessity of having two standards—one for fine, and one for wet weather.

It is not, however, necessary to trace in this way the course of all feeders. Having once ascertained the nature of the bacterial contents of two or three pure feeders, it will be sufficient to analyse the water of the various feeders as they enter the impounding reservoir, and of the reservoir or reservoirs into which all the feeders ultimately enter.

If the number of bacteria and of species of bacteria found in the feeders as they enter the reservoir is markedly greater than in the uncontaminated feeders, this will be an indication that in its downward course the feeder has become contaminated. I have on several occasions had an opportunity to test this point, and found that such a conclusion was justified by facts.

Thus, to give only one instance, I found that water taken from the mouth of a feeder, reputed absolutely unpolluted and perfectly pure, contained three or four times more bacteria than I had expected from the examination of other pure feeders in the same watershed. Notwithstanding the incredulity with which my statement was received when I said that the water must be polluted, I decided to explore the stream.

It was found that some distance above the point where the specimen had been taken an open drain poured liquid manure directly into the stream, and that on one of the banks of the stream there was a plot of land heavily manured. Owing to the configuration of the ground these sources of pollution were hidden from view, and had escaped the notice even of the local authorities. As the number of bacteria was not excessive, and as the water was chemically fairly good, I would have probably overlooked this source of pollution had it not been for my having obtained previously reliable standards.

Having thus sketched the principles, I will now describe in more detail the methods which seem to me most suitable to apply in practice, and give an account of some of the results obtained in the course of some extended investigations.

1. *Collection of water for bacteriological examination.*

It is impossible to overrate the importance of obtaining samples of water representing truly the state of the water under investigation. The number of bacteria present in water under natural conditions may, artificially, be made to vary to such an extent, that in order to obtain comparable results an observer must use exactly the same method of collection in taking samples of water from whatever source he may have to obtain them. He must therefore see that the methods he adopts are

not only suitable for obtaining water from mains or reservoirs, but also from places such as land drains, springs, brooks, pools, where the water forms shallow sheets lying on, or running over, a bottom which is often rich in micro-organisms, and the disturbance of which would modify the results of the analyses. The sediments which are to be avoided are not essential constituents of the water. Even during stormy weather they are only partly disturbed.

They are produced by the gradual sedimentation taking place as the water passes over the bed of streams or conduits, and are the effects of a natural process of purification constantly taking place in running streams, a process which can be taken advantage of artificially with great benefit.

I have carefully investigated the changes taking place in pure feeders under the influence of heavy rains, in order to ascertain how much the bacterial contents of the water might be increased by the disturbance of the silt and banks. I selected very shallow, natural, unpolluted brooks, in which the depth of water varied from ½ to 3 inches. Water collected from such brooks, and containing during fine weather a number of bacteria varying between 34 and 83 was found to contain 210 to 280 bacteria after and during heavy rains. Examples :—

	Dry weather.		Rainy weather.
A. ...	83 bacteria per c.c.	...	210 to 280 bacteria, 3 observations.
B. ...	41 to 76 „ „	...	280 „ „ „

Specimens of soil were taken from one of the banks of one of these feeders at a place where pollution of any kind was impossible owing to the land round it being absolutely free from cattle and away from any human habitation or traffic. That part of the bank was also overhanging in such a way that nothing could have been deposited on it from above. This soil was found to contain during rainy weather at least 24,600 bacteria in one gramme. Any disturbance of that soil on taking the samples of water would have therefore rendered the examination of the water perfectly useless.

If specimens are collected in bottles (even bottles as small as can be allowed for practical purposes) it is often practically impossible to obtain the water without disturbing the sediment. It is not always possible to find a slight fall or a hollow suitable for the use of such collecting vessels. This disturbance, which is so objectionable from the bacteriological point of view, is, I think, equally objectionable in connection with chemical analyses. By disturbance of shallow streams, when large quantities of water have to be collected, much vegetable organic matter is often taken with the samples; this organic matter is not normally present. The effect of this is the finding of an excess of albuminoid ammonia and oxygen consumed. (In such cases the free

ammonia and chlorine are low, and sometimes very low, showing the absence of excrementitial pollutions.)

If a bacteriological survey of a gathering-ground be undertaken, it is therefore necessary to adopt other methods than those usually employed in collecting samples from reservoirs and mains.

The method I have invariably used for the last six years is one originally introduced by Pasteur in 1860* for somewhat different purposes, and adopted since by several other observers. Tubes made of moderately thick glass $\frac{3}{4}$ inch in diameter are closed at one end like test tubes. The other end is drawn to a point, as shown in fig 1.

These tubes (A) measure from 7 to 8 inches in length, the wide part occupying about 4 inches. They can hold about 20 cubic centimetres of water. They are prepared from tubing which has never been used and has been thoroughly cleaned. The tube is sterilised by dry heat at the time of making. The drawn end forming the neck measures about $\frac{1}{2}$ inch in diameter; about 1 inch of the end is further drawn so as to be reduced to $\frac{1}{30}$ inch in diameter. The tube being so prepared is, after being allowed to cool, gently warmed again, and its open point immersed in pure water. As the tube cools, water is drawn into it on account of the contraction of the cooling air. When about 1 cubic centimetre of water has been thus sucked in, the point is removed from the water.

The water is then boiled rapidly in the tube until it has practically all been reduced to steam; the point is then quickly sealed in the flame of the blow-pipe, the steam still escaping (the flame heating the water is of course removed as soon as the sealing process has begun). The tube is then introduced into a cylindrical brass case, fitting it exactly; this case is loosely closed with an india-rubber cork [fig. 2 (3, 4)]. Each brass case used is marked to make identification easy. The brass cases, with the tubes contained in them, are sterilised in the autoclave. On removing them from the steriliser the cases are closed by pressing the india-rubber corks firmly in; this is usually rendered unnecessary by the effects of cooling. These tubes in their cases are now ready for collecting specimens. It will be noticed that it is practically impossible for contamination, even of the external surface of the tubes, to take place so long as they remain in their cases. It will also be noticed that the use of any chemical disinfectant is carefully avoided. When one of these tubes is to be used, a place is selected where the water is deep enough to allow the sealed point to be placed at least $\frac{1}{2}$ inch below the surface of the water (whenever possible, 1 inch). The point of the tube should also be at least

---

* Pasteur's bulbs were different in shape and not well adapted for the collection of samples of water.

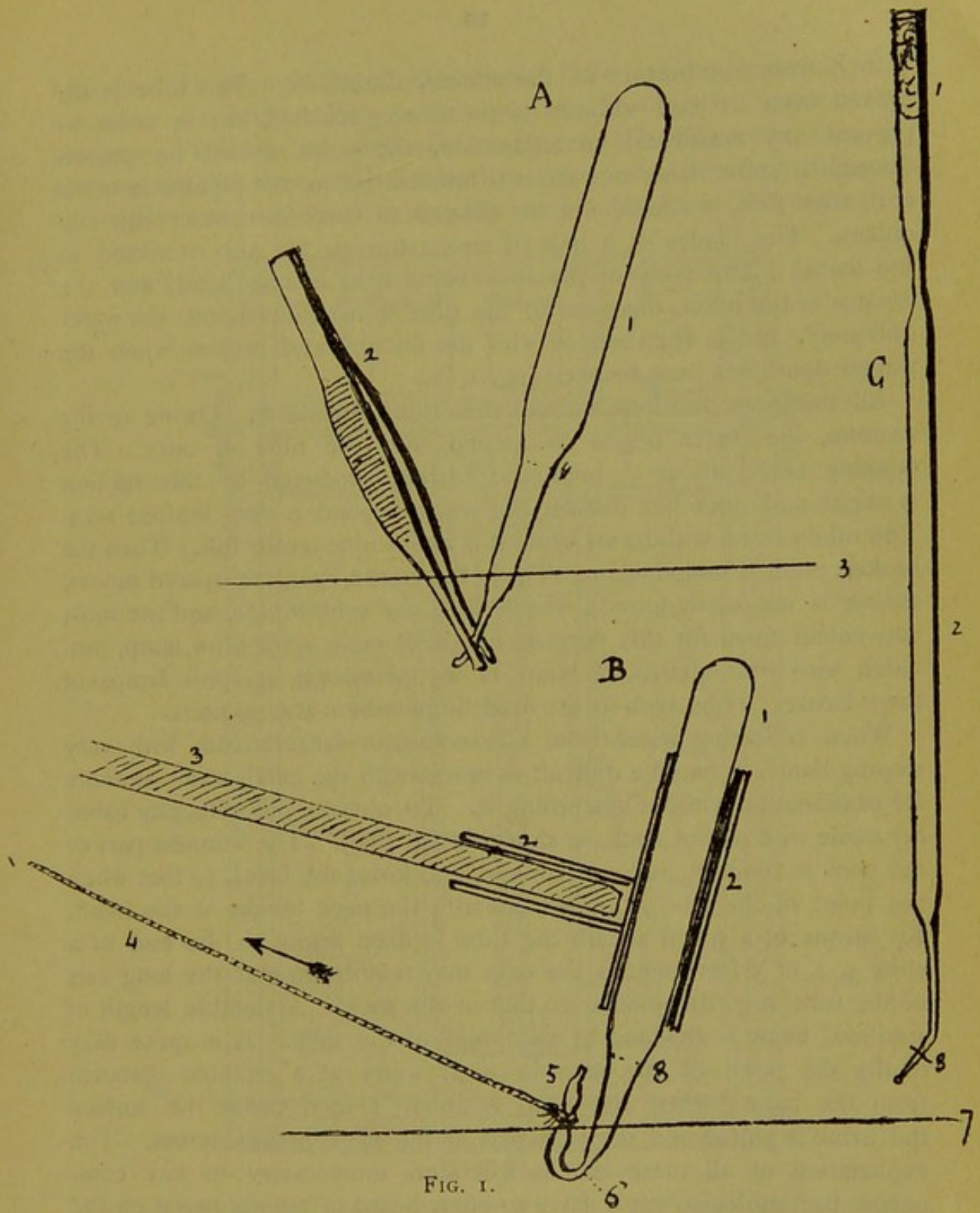


FIG. 1.

VACUUM OR SUCTION TUBES FOR COLLECTING WATER FROM STREAMS, RESERVOIRS, &C.

A. Simple form to be used when the water can be reached by hand.
1. Body of tube holding about 20 centimetres. 2. Pair of strong forceps. 3. Level of the water. 4. Place where the tube is cut open for taking water out with the measuring pipette C.

B. Tube with bent neck for taking water at a distance of several feet from the banks of a reservoir or stream.
1. Suction tube. 2. T-shaped metallic sheath holding firmly together and at right angles the tube and the end of a stick (3). 4. Sterilised thread used for pulling point (5) of the tube, so as to break its neck at the constricted part (6). 7. Level of the water. 8. Place where tube is opened to take water out with measuring pipette C.

C. Measuring pipette used for taking water out of suction tubes.
1. Mouthpiece. 2. Body, holding about 3 centimetres. 3. Place where the bent point is broken across. When used for counting, this pipette is held horizontally, the bent point being turned downwards; the pressure under which the water flows is thus kept constant.

½ inch from the bottom of the stream, drain, &c. The tube is removed from its case without its point being touched, but in order to prevent any accidental contamination, the point should be passed through a spirit flame one or two minutes before the pipette is used, and, after that, it should not be allowed to come in contact with any object. The blades of a pair of strong forceps are also sterilised in the flame. The body of the tube being held in one hand, and the forceps in the other, the point of the tube is introduced into the water obliquely, and is then seized with the forceps and broken when the proper depth has been reached (fig. 1, A).

All this must be done without disturbing the water. Owing to the vacuum, the water begins to ascend into the tube at once. The opening being about $\frac{1}{30}$ inch, the current produced by this suction is slight and does not disturb the water beyond a very limited area. The tube can be withdrawn when it is about nine-tenths full. Then the broken point is sealed again. To do this in the open (on upland moors, shelter is not easily found), it is well to use a blow-pipe, and the most convenient form for this purpose is a self-acting spirit blow-lamp, provided with wind-guards, or some of the petroleum or spirit lamps of the "Roarer" type, such as are used by plumbers and painters.

When collecting water from a reservoir, or large stream with very sloping banks, it may be difficult to reach, with the hand, water suitable for examination without disturbing it. To obviate this difficulty tubes are made with a bent neck, as shown in fig. 1 (B). The thinnest part of the neck is about $\frac{1}{30}$ inch in diameter, and forms the bend, so that when the point of the tube is pulled laterally the neck breaks at the bend. By means of a metal sheath the tube is fixed firmly to the end of a stick 3, 4, or 5 feet long, as the case may require, so that the long axis of the tube is perpendicular to that of the stick. A suitable length of sterilised twine is attached to the point of the tube. It is quite easy to dip the point of the tube in clear water at a suitable distance from the bank; when the bend is about 1 inch below the surface the twine is pulled and the thin part of the neck breaks across. The explanation of all these details will seem unnecessary to any competent bacteriologist, yet I have so often heard of reports based on the examination of water collected in very different ways, that I wish to leave no room for doubt with regard to the precautions which seem to me necessary.

I specially object to the use of boats for the purpose of collecting samples of water by means of bottles. The use of a boat would not make the precautions I have described unnecessary, as the hand cannot possibly reach a place which has not been affected by the motion of the boat, except when one has to deal with a rapid stream.

It is somewhat difficult to fill a vacuum tube direct from a tap or

hydrant. To get over this slight difficulty I always provide myself with a number of small so-called "self-closing tins," or any other vessel easily sterilised. The tins are thoroughly washed with boiling water, then by pressing the lid they are hermetically closed; they are then wrapped up in paper and sterilised thoroughly in the autoclave. When one of

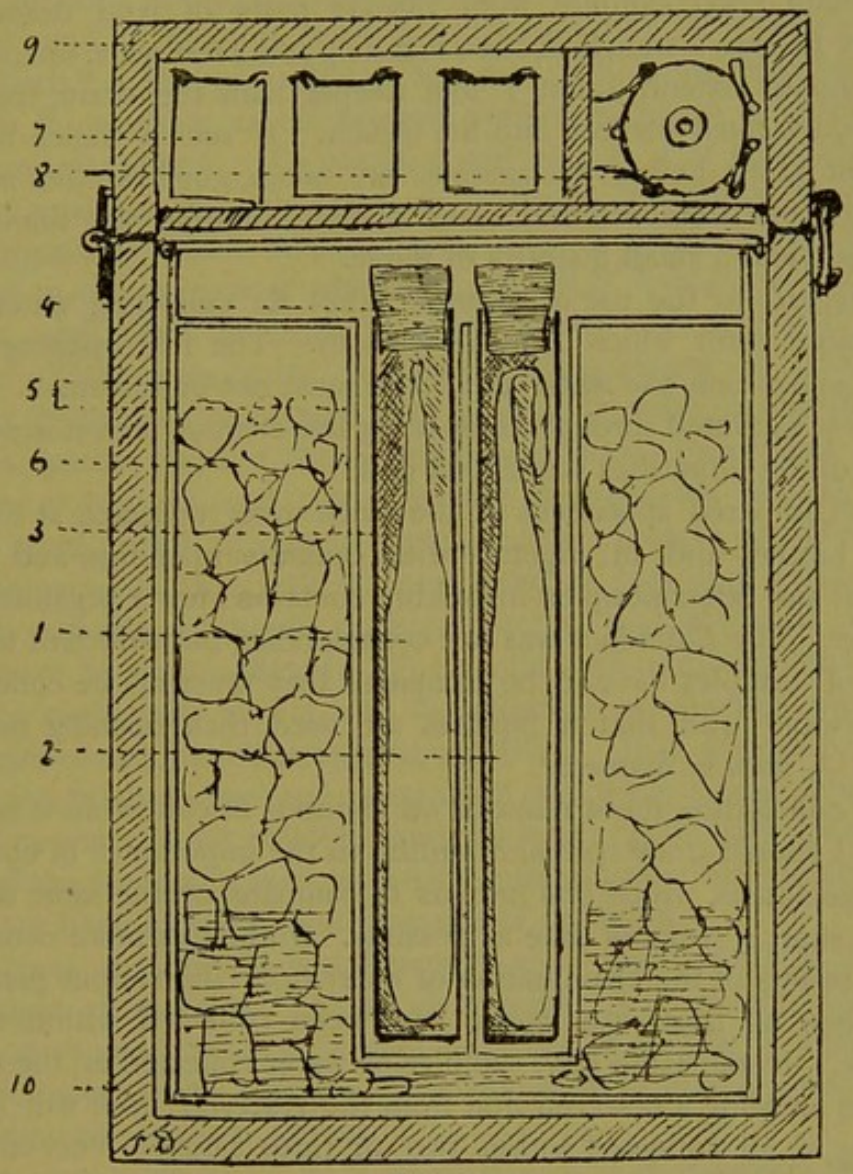


FIG. 2.

OUTFIT FOR COLLECTING SAMPLES OF WATER FOR BACTERIOLOGICAL ANALYSIS.

Transverse section of portable case to hold twelve collecting vacuum tubes, and to keep them in ice.

1, 2. Vacuum or suction tubes (*i.e.*, elongated form of Pasteur's suction bulb, 1860).
1. For collecting water within reach of the hand. 2. For taking water at a distance from a bank. 3. Metallic case for above. 4. Cork for case. 5, 6. Zinc ice-chamber. 7. Tins sterilised and hermetically closed for the collection of water from taps, hydrants, &c. (used in conjunction with 1). 8. Self-acting spirit blow-lamp with wind-guards. 9. Lid of wooden case.

these tins is to be used, the paper is unwrapped without the lid being touched; for further precaution the lid is passed two or three times in the flame of a spirit lamp, then it is levered out by means of any flat piece of metal, which must be thoroughly sterilised before use. The

tin is filled at once, and from it a sample is taken in a vacuum tube in the same way as has been previously explained. With regard to the collection of water from taps or hydrants, I have found it desirable to allow the water to run freely for twenty minutes or half an hour before the specimen is taken.

To obtain good samples from various parts of land drains it is necessary to have a trench, about 2 feet long and 2 feet wide, opened; this trench should extend about 1 foot deeper than the drain, the upper end of which must project into the trench. If such a trench be made forty-eight hours before the samples are taken, good samples are easy to collect, both from ordinary stone drains and from pipe drains, even when they yield a small quantity of water.

With regard to the use of vacuum tubes for collecting water, there is one more point which I must refer to. The fine opening of the tube through which the water is sucked must not be too small. When the water is aspirated through an opening less than $\frac{1}{30}$ inch it is deprived of much of the impurities suspended in it.

I have compared specimens of the same water collected at the same time in bottles and in pipettes with capillary openings, and I have found that the latter samples invariably contained fewer organisms than the former. The difference was not considerable, but sufficient to show, 1st, that if samples have to be compared they must all be collected in the same way. 2nd, that if pipettes are used, their opening must not be under $\frac{1}{30}$ inch in diameter.

Before concluding these remarks on methods of collection it is necessary that I should draw special attention to the importance of collecting all the specimens, which one intends to compare, on the same day and within as short a space of time as possible. Nothing is more convincing than the results of the examination of water from the various parts of a feeder when all these specimens have been collected within one or two hours. In the same way, samples of water collected at the mouths of feeders entering a reservoir and from the reservoir itself will be very instructive if all collected within two or three hours. Very different will be the case if all these samples have been collected on different days, or even when some have been taken early in the morning and others late in the afternoon, especially when the state of the weather has changed meanwhile.

I think it desirable to reduce to a minimum the time spent in collecting samples, and that alone should be enough to indicate the inadvisability of making plate cultures on the spot. There are, however, still better reasons for objecting to that kind of procedure.

2. *Methods of cultivation.*

The two most important objects which the hygienist has before him when he undertakes to make a bacteriological examination of water are—

(1) To ascertain whether there is evidence of pollution with excrementitial products.

(2) To find out whether there are pathogenic germs present.

To the naturalist a study of the bacterial flora of water coming from different districts is undoubtedly fascinating, and judging from results I have obtained in that direction during the last six years in Manchester and surrounding districts, such a study may not be without practical applications.* The two objects, however, which the hygienist has in view are those which at the present time must guide us chiefly in the selections of method. To obtain these objects it is not necessary to conduct analyses in such a way as to insure the discovery of all the micro-organisms which may be present in a given water, but it is essential to discover those which are *capable* of living freely on animal matters, and, when possible, of those which are *capable* of causing disease. The presence of excrementitial pollution or decomposing organic products is associated with a large increase in the number of bacteria, partly because those products contain themselves immense numbers of bacteria, and partly because they provide suitable material for the rapid growth of certain bacteria always present in water. Some of these bacteria thrive well in water, even in very pure water, but they multiply exceedingly rapidly in water such as that of drains or streams which have become polluted. The actual number of bacteria, whether these be pathogenic or not, can therefore be used as an indication of the presence or absence of organic pollution. It is, of course, necessary to know exactly the normal contents of any water under investigation, when this water is not polluted, and this can only be done by examining some of the feeders in each gathering-ground under investigation before they have been exposed to any chance of pollution.

Evidence of pollution is sufficient to show that a water is suspicious, and might at any time become dangerous; it is enough that specific pollution should have been *proved* in a certain number of cases to have been connected with excrementitial pollution to *establish* the reality of the danger of sewage pollution.

In current work it will be therefore enough to demonstrate the existence of undoubted pollution to establish the fact that a water is potentially, if not actually, dangerous to health.

This being the case, it is evident that the methods used must give the means of finding the bacteria present in any water and capable of growing on animal matter.

The methods need not show whether these bacteria are *saprophytic* or *pathogenic.*

Among the bacteria found in decomposing animal matter many are

* Such a study implies the use of several methods of cultivation not commonly employed.

saprophytic, and do not grow so well on special media at the temperature of the body as they do in alkaline nutrient gelatine at the temperature of 22° C. On the the other hand, the pathogenic bacteria which it would be of importance to find in water, such as the bacillus of typhoid fever, the bacilli allied to the bacillus coli, the spirillum of cholera, the bacillus anthracis, all grow well in four days in alkaline nutrient gelatine at temperatures ranging between 21° and 22° C. All the bacteria which it is usually important to reveal can therefore be grown on nutrient gelatine, and therefore that medium is the most suitable one to use for the purpose of *estimating the number of bacteria as an indication of the presence or absence of pollution.* Nutrient gelatine is also indicated by the fact that, since its introduction by Koch, it has been used by the large majority of bacteriologists in water examinations.

It is desirable therefore to follow accurately the directions given by Koch for its preparation, but as he has given no exact data with regard to the degree of alkalinity, it would be well if a certain standard were generally adopted. I have myself since 1894 used the following method, which is based on the researches of Reinsch :—

The nutrient gelatine is prepared according to Koch's direction (the following ingredia being used in the preparation : 1,000 c.c. of water, 500 grammes of beef, first quality, 10 grammes of dry peptone, 5 grammes of common salt, 100 grammes of white gelatine). Then the acid medium is neutralised with normal solution of caustic soda according to Schultze's method, phenolphthalein being used as the indicator. After this, 1 gramme of pure carbonate of sodium is added to each litre of medium. Even when all the necessary precautions are observed, some slight variations occur in the quality of the medium. These are chiefly due to the impossibility of obtaining meat, peptone, and gelatine of constant composition.

Notwithstanding these unavoidable slight variations, all aërobic, pathogenic, and putrefactive bacteria usually found in polluted water grow rapidly in this alkaline nutrient gelatine.

The plates should be made, I need hardly say, in glass capsules, generally known as Petri dishes.

Auxiliary methods of cultivation may also be used ; and after trying more especially the use of agar and alkaline peptone bouillon cultures incubated at 37° C., and of acid nutrient gelatine incubated at 22°, I have come to the conclusion that the most useful results are obtained by one or the other of the following methods :—

(*a*) The water itself is incubated for some twelve or eighteen hours at 37° C., and then the number of bacteria is estimated in the ordinary way by plate cultivation. In polluted water containing an excess of organic matter an extremely rapid multiplication of bacteria is observed. In unpolluted water containing only water bacteria and a very small

amount of organic matter very little or no multiplication takes place, and the growth of the water bacteria liquefying gelatine is checked to a remarkable extent.

(*b*) To 5 c.c. of alkaline bouillon 1 c.c. of the water is added, and the tubes are incubated at 37° for twenty-four or forty-eight hours. Polluted waters usually give rise to an abundant growth, causing a general cloudiness and often the formation of a thick pellicle on the surface of the fluid. Unpolluted waters produce at most a doubtful cloudiness, which barely impairs the transparency of the medium.

(*c*) Animals may be inoculated with water incubated or not. This, however, I have not found yet a very useful method, and I would reserve it for special cases only.

I will give, further on, results obtained by each one of these methods, and show their application in practice.

With regard to the quantity of water which must be used and the ways of measuring it, there are some important details to keep in mind. It is only when waters are very pure that such quantities as 1 c.c. and $\frac{1}{2}$ c.c. can be used for counting, *after four or five days' incubation at* 21° *or* 22° *C.* Even in slightly polluted waters there are so many liquefying bacteria, that after the second or third day the plates would be useless. It is therefore necessary, when testing a water of unknown quality, to prepare plates with various quantities of water.

The quantities I have found most suitable for ordinary waters have been $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{10}$ c.c. When the water is probably polluted I use $\frac{1}{4}$, $\frac{1}{20}$, and $\frac{1}{50}$ c.c. When the water is very polluted, it is seldom possible to use more than $\frac{1}{100}$ or $\frac{1}{200}$ c.c. with any hope of success. When quantities under $\frac{1}{20}$ c.c. have to be used it is of course necessary to dilute the water under examination with sterilised water, so as to make it possible to measure accurately the amount wanted. On the other hand, it is possible to obtain quantities above $\frac{1}{20}$ c.c. by the following method:—With the pipette represented in fig. 1 (C) water is taken out of the collecting tube. Owing to the shape of the measuring pipette, it is possible to hold it *almost horizontally* and to allow drops to fall slowly from its fine point. This point is of such a size that it will deliver about 50 or 60 drops to the cubic centimetre. The size of the drop is ascertained by finding the number of drops contained in 1 gramme of water weighed accurately on the chemical balance.

Suppose 58 drops have been found to weigh just under 1 gramme, and 59 just over, we will say that each drop is the $\frac{1}{58}$ of 1 gramme, plus a negligible fraction. If about $\frac{1}{2}$ c.c. of water is required, 29 drops of the water delivered from that pipette will give the quantity wanted; the nearest quantity to $\frac{1}{4}$ will be 14 drops and the plate will be marked $\frac{14}{58}$, from which fraction it will be easy to calculate the contents of 1 c.c.

If about $\frac{1}{10}$ is wanted, then 6 drops will be taken and the plate will be marked as having been made with $\frac{6}{58}$ c.c.

For each sample of water examined a fresh sterilised pipette is used, so that there can be no extraneous germs introduced.

I was led to adopt this method owing to the difficulty of measuring accurately small quantities of water with the ordinary graduated pipettes in use, and in order to avoid the necessity of diluting the water investigated with sterilised water. Dilution implies a number of measurements and the use of additional vessels. Each manipulation adds to the chances of contamination, and dilution methods should therefore be avoided whenever it is possible to do so. When bacteria are few in number, plates made with various quantities of water measured by the method I have described give concordant results, the tendency being generally for the small quantities, such as $\frac{1}{10}$ and $\frac{1}{20}$, to lead to higher estimations than the larger quantities. This is partly due to the effects of calculation, which multiplies the errors of observation, and also to the fact that when liquefying bacteria are few they do not interfere with the growth of other bacteria.* When colonies are not numerous they reach a large size and are more distinct. When the bacteria are very numerous, 5,000 or 10,000, or more, the discrepancies are more marked but then they become unimportant. These errors are common to all methods of measurement, and are distinctly diminished by the method I have described. I make it a rule to count three plates prepared at the same time with various quantities of the same water, and I think results obtained from one plate alone are not reliable. I need not say that all the usual precautions must be taken to sterilise by heat everything that is likely to come in contact with the water at any stage of the manipulations described above. It is not till the fourth day of incubation at 21° to 22° C. that the number of colonies ceases to increase rapidly from day to day. After the fifth day no important increase in number takes place in plates containing less than 100 colonies to the whole plate (Petri dish). The plates made with 1 and $\frac{1}{2}$ c.c. should invariably be counted on the second, third, and, if possible, fourth day, to estimate the number and kinds of quickly liquefying colonies. The plates made with $\frac{1}{4}$, $\frac{1}{10}$, $\frac{1}{20}$, and $\frac{1}{30}$ c.c. should be counted on the third, fourth, and fifth day, to count the non-liquefying and slowly liquefying colonies, and should be again examined at the end of one week, and even ten days (for they are seldom entirely liquefied), to discover if any delayed growth has taken place, and to find out the characters of some kinds, such as the bacillus violaceus, streptothrix brunneus, &c., which are seldom evident at the end of five days. After the fourth or fifth day the plates may be with advantage kept out of the incubator. In the

* Certain bacteria also inhibit or favour the growth of others.

case of *very polluted water*, when the numbers estimated have reached 5,000 or 10,000, *accurate numeration is a matter of secondary importance.* Two or three days' incubation at 22° are generally sufficient to establish the bad qualities of these waters, and further observation is necessary only to find out the kinds of bacteria present.*

3. *Time and place for the preparation of plate cultures.*

Much has been said on the importance of preparing the plates immediately after the collection of samples of water. Many bacteriologists prepare their plates on the spot where the water is collected, and bring them back to the laboratories after the gelatine has set. Much might be said in favour of that method when the only thing in view is the testing of water as collected in houses or at waterworks. But such examinations, except for the purpose of testing the action of filter beds, are of limited use, and when a regular bacteriological survey of a gathering-ground is undertaken it is impossible to observe in the field the precautions necessary to insure accuracy.

As the water has to be kept in some cases before examination in the laboratory, it should be kept for the same length of time in all cases, if one wishes to obtain comparable results. Fortunately this can be done, with practically no alteration of the samples, by packing in ice. I have above described how the collecting pipettes are placed in their cases. These can be conveniently packed in ice in a portable refrigerator. The refrigerator I use for this purpose is represented in fig. 2, which will explain itself.

The samples are placed in this box immediately after being collected, and are not removed from it till the moment they are wanted for making the plates. Bacteria do not increase in number under these conditions, the temperature remaining invariably under 2° or 3° C. The plates should always be made within a few hours of the time of collection, even when these precautions are taken, as there is a tendency to a slight diminution in the number of water bacteria when samples of water are kept in ice and in hermetically closed vessels.

4. *Counting of bacteria.*

No counting can be considered of any use for the purpose of proving that a water is good unless the plates have been incubated for four or five days at least at 21° to 22° C. The counting of kinds requires a

* Determination of the species of water bacteria is a matter of great difficulty in some cases. This is partly due (1) to the influence which some water bacteria have upon the mode of growth of others, growing in the same plate side by side with them; (2) to the rapid alteration which take place in the cultural character of many water bacteria after they have been isolated from water and grown for some time on various nutrient media.

longer time still ; plates should be examined from time to time, whenever possible, for a fortnight. It is, however, unimportant to know the kinds of bacteria which grow very slowly in gelatine ; for purposes of comparison, it is enough to find out all the kinds *clearly distinguishable from each other*, and which can be detected in the course of one week. I think it is desirable, when the number of colonies is not excessive, to invariably count the colonies present in the whole plate, and not to estimate the total amount by counting the numbers present in a fraction of the plate. When colonies are very numerous an approximate estimation will be quite sufficient. Three plates made with various quantities of water should be counted in each case, and the number of bacteria in the cubic centimetre obtained by taking the average between the three observations.

5. *Observations relating to methods used.*

One must be prepared for rather wide margins of experimental error in bacteriological analyses.

The methods I have described previously have for their object to reduce this error to a minimum and to make it affect results, as far as possible, always in the same direction, so as to render them comparable.

It must, however, be admitted that absolutely concordant results are difficult to obtain, and it is therefore necessary to know the limits within which comparisons are justifiable.

The chief source of difficulty is the *unequal distribution of bacteria in water*. It is almost impossible to obtain water from streams, reservoirs, filter beds, mains, &c., entirely free from minute flakes of organic débris ; these are often so few, so small, and so translucent as to be practically invisible to the naked eye. Their presence can, however, be demonstrated by the microscopical examination of sediments easily obtained by centrifugalisation or filtration through a Chamberland porcelain filter. To these small flakes bacteria are attached in variable numbers ; some of the flakes indeed are entirely composed of microbes.

When small quantities of any water are measured for analysis, it is evident that if a few more of these flakes find their way into one of the measured quantities than into the others, this will give rise to an increase in the number of bacteria found in that specimen. It is evident that the discrepancies produced thus must be proportionally greater when the quantities of water used for making the plates are very small.

This source of error is considerably diminished by shaking violently the water in the bottles or tubes in which it has been collected. This should be done *immediately before measuring the water* previous to making the plates.

But even after thus breaking up the flakes as far as possible and mixing the water thoroughly with the melted gelatine, a few bacteria remain almost invariably clumped together.

This is proved by the occasional grouping of colonies which is not unfrequently observed in very well-prepared plates.

It is also made evident by what sometimes occurs when one isolates bacteria from a plate for the purpose of obtaining pure cultures.

Every bacteriologist has found that tube cultures prepared directly from small, discrete, sharply defined colonies may occasionally be found to contain two or even three species instead of one. This is specially the case when the plate has been incubated for only two or three days only, and all the bacteria have not had time to produce visible colonies. (When proper precautions are taken it is quite easy to exclude chances of accidental contaminations.)

It is chiefly this unavoidable source of error which makes it desirable to prepare at least two or three plates with each sample of water examined, and to take the mean of the results obtained with these three plates.

I will now give a few examples showing the influence which some of these technical difficulties have on the results of bacteriological examinations of water.

A. *The multiplication of bacteria taking place in samples of water kept at the temperature of inhabited rooms* has been shown by various observers to be very rapid.

At least three factors influence the rate of multiplication. 1st, the kind of bacteria present; 2nd, the proportion between the various kinds; 3rd, the amount of organic and inorganic matter present in the water.

One should therefore expect to find differences in the increase of bacteria taking place in waters of various sources.

I had a good opportunity to test this point in 1894, whilst investigating the Manchester water supply.

Samples of water were obtained from ten hydrants situated in various parts of the town, and connected with five reservoirs, which I will call here A, B, C, D, E.

Owing to the great distance between some of the hydrants, and to the large number of plates prepared, I was not able to allow the same interval of time between the collection of the samples and the preparation of the plates in every case. Moreover, the weather being very cold (winter), it was not thought necessary to keep these samples in ice. The following figures, however, clearly suggest that some multiplication had taken place in the specimens which had been kept three or four hours; this would have been entirely prevented had they been kept in ice.

TABLE I.

Water collected in November and December from mains situated in nine different town districts.

Date.	Service Reservoir.	Main.	Size of Main.*	Pressure as tested at the time.*	Time between collection and plating.	Number of bacteria in 1 c.c. after four days' incubation at 21° C.	Water kept three days at 17° and 18° C. Number of bacteria found in 1 c.c. after four days' incubation.	Fraction indicating increase of bacteria after three days at room temperature.
			Inches.	Pounds.	Mins.			
Nov. 10th, 1894...	A	1	3	53	295	69	...	...
		2	8	49	290	61	...	...
" " ...	B	3	7	55	280	62	...	...
		4	7	33	260	75	...	...
" " ...	C	5	9	24	250	50	...	...
		6	6	45	235	30	...	...
" " ...	D	7	8	34	205	33	...	...
		8	8	55	140	55	...	...
" " ...	E	9	3	52	140	34	...	...
		10	Private main a few pounds		5	17	...	...
Dec. 1st, 1894 ...	A	1	3	58	105	29	24,600	$\frac{848}{1}$
" " ...	B	3	7	55	95	29	32,000	$\frac{1103}{1}$
" " ...	C	5	9	26	85	44	49,000	$\frac{1113}{1}$
" " ...	D	7	8	30	50	34	44,300	$\frac{1303}{1}$
" " ...	E	9	3	47	50	12	310,000	$\frac{25833}{1}$

* The pressure of water and size of main are given to show that they did not influence materially the number of bacteria; the length of the mains, on the other hand, has a distinct influence. This will be shown later on.

It is evident from this table that the water from reservoir E was more liable to deterioration than the water of the other reservoirs. Such a deterioration would take place readily whenever that water was kept in house cisterns situated, as usually they are, in a warm place to prevent freezing.

Reservoirs B, C, D, E, received their supply from the same gathering-ground; moreover, before reaching C, D, E, the water had to pass through the higher reservoir B. There must have therefore been something peculiar to the service reservoir E to account for the tendency which its water had to deteriorate.

I found on inquiry that this reservoir was liable to changes, which had attracted the attention of the officials in charge.

Thus, when the atmosphere was very calm, a penetrating smell, described as that of "stale water," had very often been noticed. On one occasion, during a very hot day, the water was rendered turbid

over a surface of about one acre by a brown cloud which seemed to rise from the bottom (this was described as "bloom"). (The limited size of this cloud and its mode of appearance suggest that some of the sediment at the bottom must have been displaced by some unusually marked current produced by differences of temperature in the various parts of the reservoir.) At such times it is evident that the water must have been loaded with organic matter, living and dead.

At the time when I examined the water it was apparently free from an excess of organic impurity (this was also found to be the case by Professor Dixon, who analysed chemically samples collected at the same time as mine were).

The only evidence, therefore, which indicated some abnormality was the rapid multiplication of bacteria taking place after storage at a temperature of about 18° C. I had no opportunity to try at that time the effects of incubation at a higher temperature.

Within certain limits, it may be said that the more organic matter (suitable for bacterial food) there is in water, the more rapid the multiplication of bacteria will be when the water is kept at a temperature of 12° to 22° or 25° C.

But, even in the presence of a large amount of organic matter, it is quite easy to check entirely this multiplication by reducing the temperature. Below 4° C. no increase takes place; on the contrary, a slight diminution may be observed, as the following observation clearly shows:—

Having obtained some fresh milk, I found that twenty minutes after milking it contained 2,929 bacteria to the cubic centimetre.

I filled two sterilised bottles, absolutely similar in every respect, with this milk. One of the bottles was left in the room at a temperature varying between 17° and 18° C., and the other was packed in ice. After four days I estimated again the number of bacteria present in the milk, and obtained the following results:—

	Twenty minutes after milking.	Four days after.	
		Milk packed in ice.	Milk kept at about 18° C.
Number of bacteria in 1 c.c.	2,929	1,512 Milk quite fresh.	∞ Milk sour.

B. In order to ascertain how far the *collection of water in vacuum tubes* might affect the estimations I made a number of observations. In Table II. I give the results of one set of experiments only. They give a fair idea of other observations of the same nature.

The samples used in this case were obtained from a private main

in Manchester. The water had been allowed to run for several hours before the specimens were collected. All the plates were prepared within one hour of the time of collection and incubated at a temperature of 21° to 22° C.

TABLE II.

Collecting vessel.	Day of counting.	1 c.c.	0·5 c.c.	Averages for each method of collection.
		Total number in 1 c.c.	Total number in 1 c.c.	
Flask.	3rd day	15	8	11½
	4th day	20	10	15
Vacuum tube.	3rd day	10	8	9
	4th day	10	12	11
Averages for both methods combined.	3rd day	12½	8	—
	4th day	15	11	

Considering the purity of this water, the results are as concordant as may reasonably be expected.

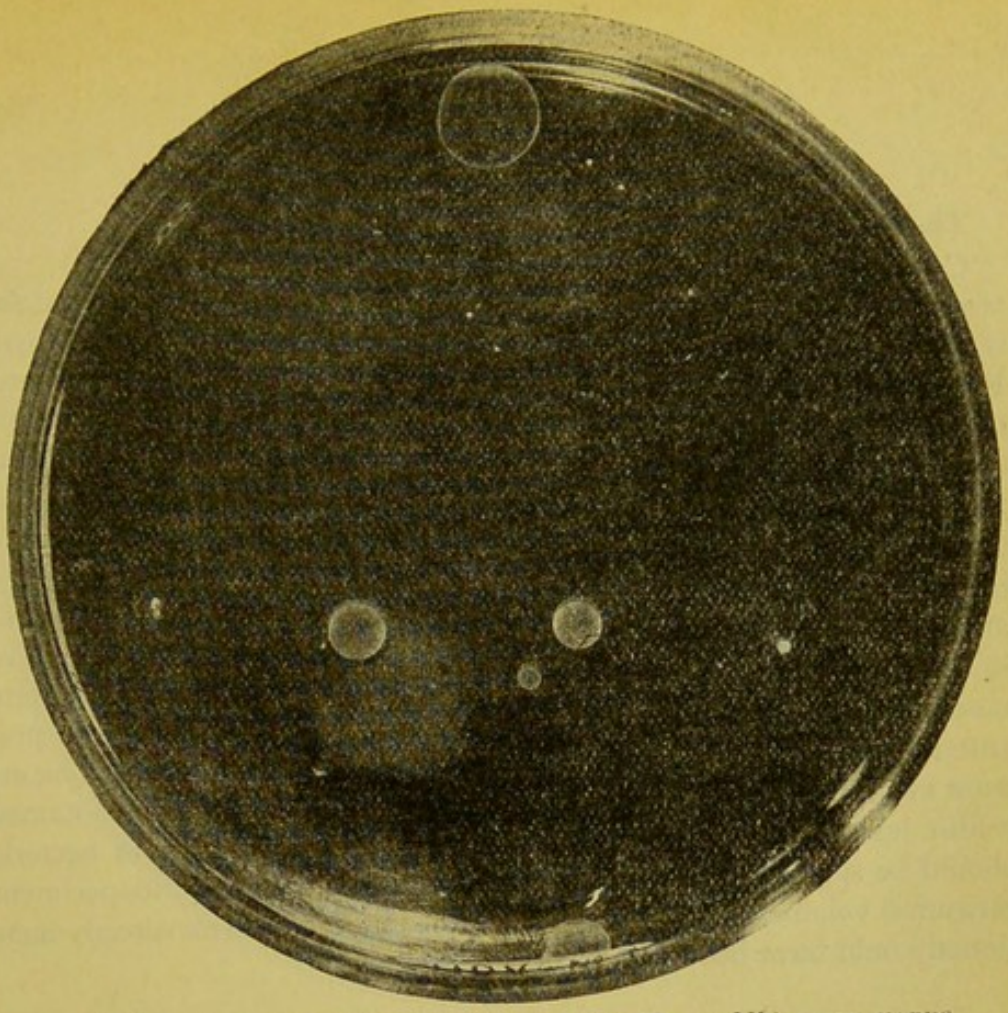
The differences due to unequal distribution of bacteria are always more marked in waters containing a small number of micro-organisms than in those containing from fifty to 500 bacteria.

That the error is due to the grouping of the microbes more than to the methods used is well shown by the proportion of various kinds of bacteria found in various measured quantities taken from the same sample at the same time.

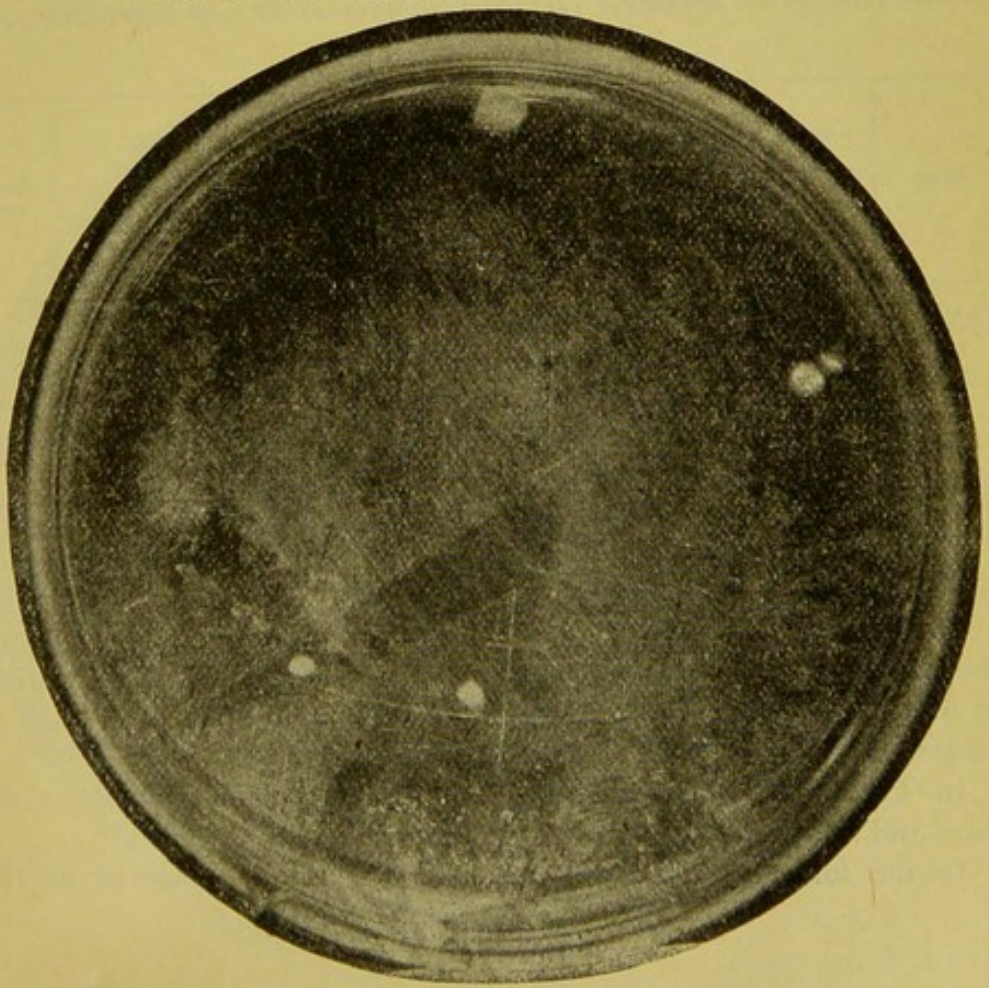
Thus in the four plates partly analysed in Table II. the following numbers of liquefying and non-liquefying colonies were found :—

TABLE III.

	Liquefying colonies in 1 c.c. (4th day).	Non-liquefying colonies in 1 c.c. (4th day).	Total.
Flask 1 c.c.	9	11	20
,, 0·5 c.c.	6	4	10
Tube 1 c.c.	6	4	10
,, 0·5 c.c.	4	8	12



Photograph 1.—WATER OF FEEDER B_3 (SEE TABLE VI.) FIFTY YARDS ABOVE FARM. WATER NOT INCUBATED. ($\frac{1}{2}$ GRAM.)



Photograph 2.—WATER OF FEEDER B_3 (TABLE VI.) AFTER INCUBATION FOR EIGHTEEN HOURS. ($\frac{1}{10}$ GRAM.)

The discordance is not always so well marked as in this case.

C. As I have already pointed out, various methods may be used for *measuring water*. When a whole cubic centimetre can be used there is no great difficulty in obtaining tolerably accurate measurements. For smaller quantities weighing is preferable to measuring by means of graduated pipettes. In the latter case a very appreciable amount of water remains on the sides and at the point of the pipette, and this forms a fraction of the water which is inversely proportioned to the quantity measured. It is not practical to wash the pipette with the melted gelatine. In Table IV. I give the number of bacteria found, after incubation for four days at 21° C., of plates prepared with five samples of Manchester water. Of each sample three quantities were measured—one by bulk and two by weight. All the plates were prepared at the same time. As the temperature at which the measurements were taken was about 18° C., one cubic centimetre of water contained a little less than one gramme of water, so that the number of bacteria should be slightly larger in the weighed specimens than in the specimens measured volumetrically, even if the other source of error already mentioned could have been excluded.

TABLE IV.

Water collected on December 1st, 1894 (number in 1 c.c. or 1 gramme given in each case).

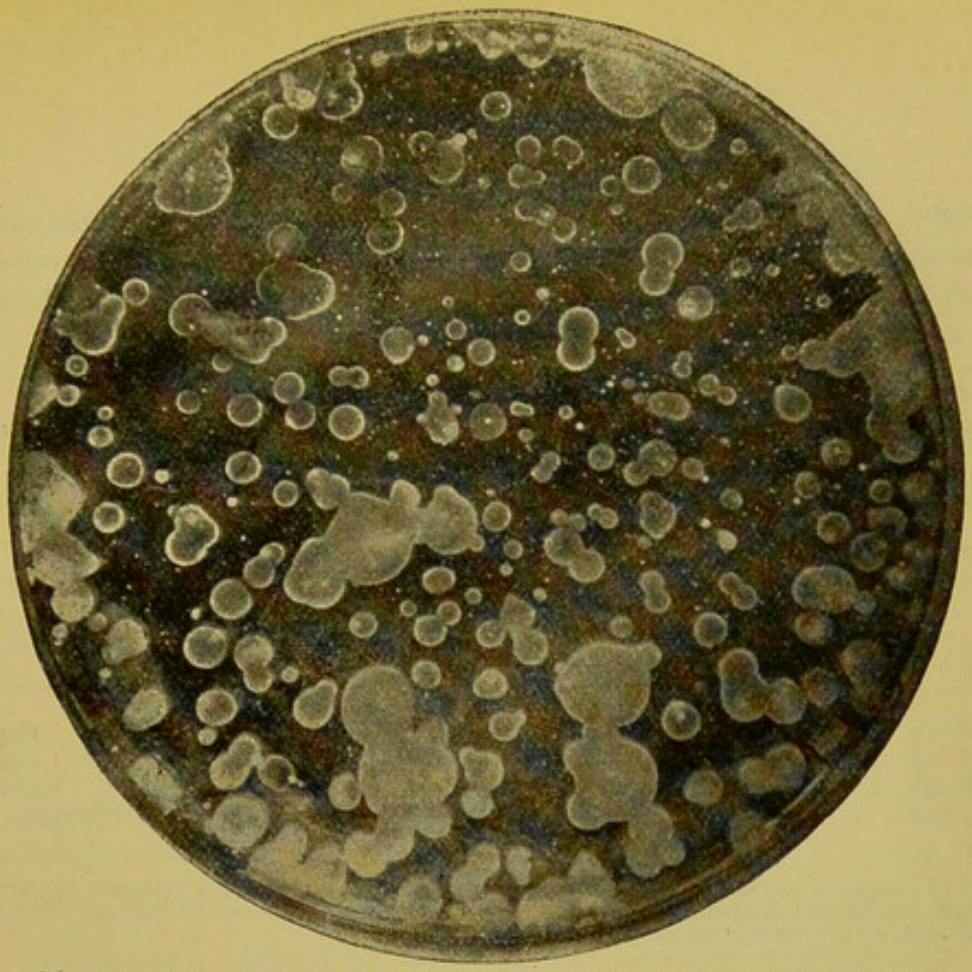
Main.	1 c.c. measured with pipette.			1 gramme.			0·5 gramme.			Average.
	Liquefying.	Non-liquefying.	Total.	Liquefying.	Non-liquefying.	Total.	Liquefying.	Non-liquefying.	Total.	
No. 1	11	12	23	14	15	29	18	16	34	29
„ 3	14	17	31	8	17	25	14	16	30	29
„ 5	26	18	44	13	16	29	26	34	60	44
„ 7	6	15	21	9	30	39	10	34	44	34
„ 9	12	1	13	9	6	15	4	2	6	12

With one exception the discrepancies are not excessive, and would have been less if the water had been less pure.

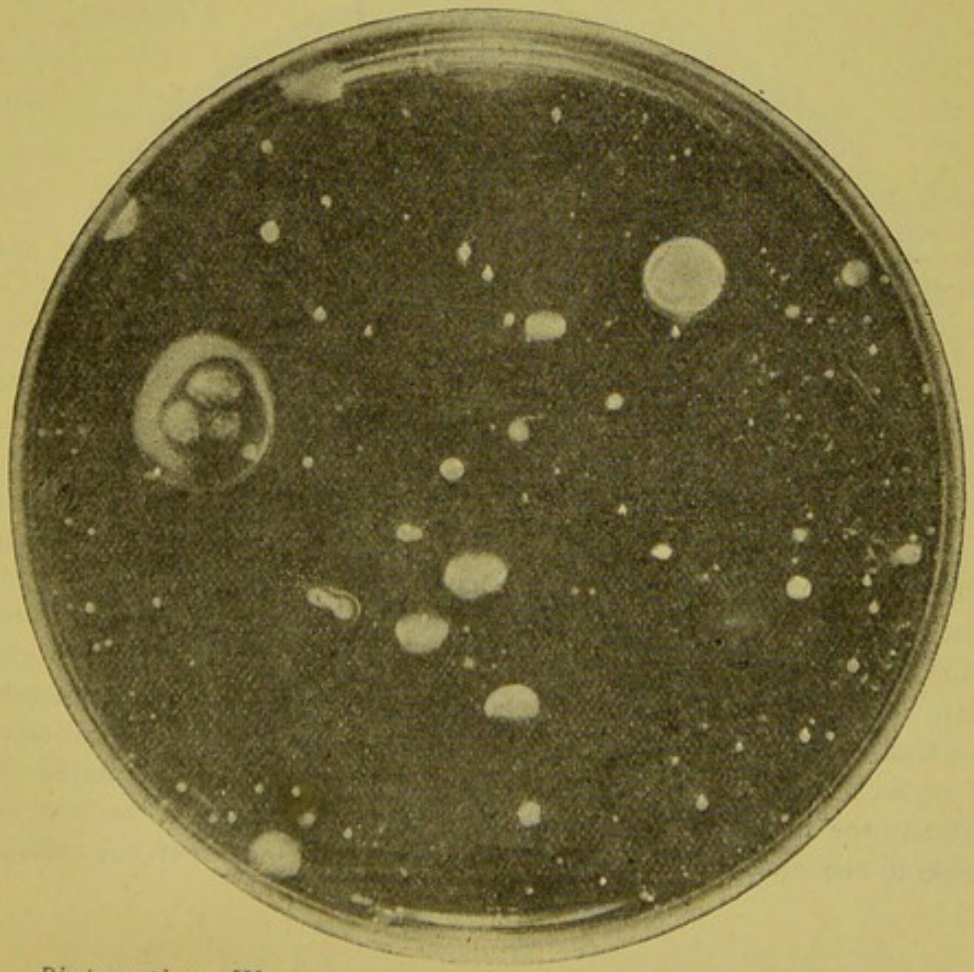
D. The *necessity of counting the number of colonies*, not only on the third but also on the fourth, and sometimes on the fifth day of incubation, at 21° to 22° C., is more evident in some cases than in others.

In Table II. I have given the numbers of colonies found on the third and fourth days in a pure water.

In the following table will be found the results obtained on the



Photograph 3.—WATER OF FEEDER B_2 EIGHTY YARDS BELOW THE FARM FROM WHICH THE DRAINAGE WHICH POLLUTED THE STREAM WAS DERIVED. WATER NOT INCUBATED. ($\frac{1}{2}$ GRAM.)



Photograph 4.—WATER OF FEEDER B_2 EIGHTY YARDS BELOW THE FARM FROM WHICH THE DRAINAGE WHICH POLLUTED THE STREAM WAS DERIVED AFTER INCUBATION FOR EIGHTEEN HOURS. ($\frac{1}{10}$ GRAM.)

second, third, fifth, and sixth days: A. with the water of a polluted reservoir, B. with the water of a main receiving the same water mixed with pure water from another reservoir. All the plates were incubated at 21° C.

TABLE V.

A. Water (B_{32}) *collected on November 30th*, 1897 (*number of colonies in 1 gramme*).

	⅛ gramme.			⅛ gramme.		
	Liquefying.	Non-liquefying.	Total.	Liquefying.	Non-liquefying.	Total.
2nd day	74	1,173	1,247	64	725	789
3rd day	501	1,312	1,813	341	1,088	1,429
5th day	Plate nearly liquefyed.			427	1,088*	1,515
6th day	—	—	—	No marked increase.		

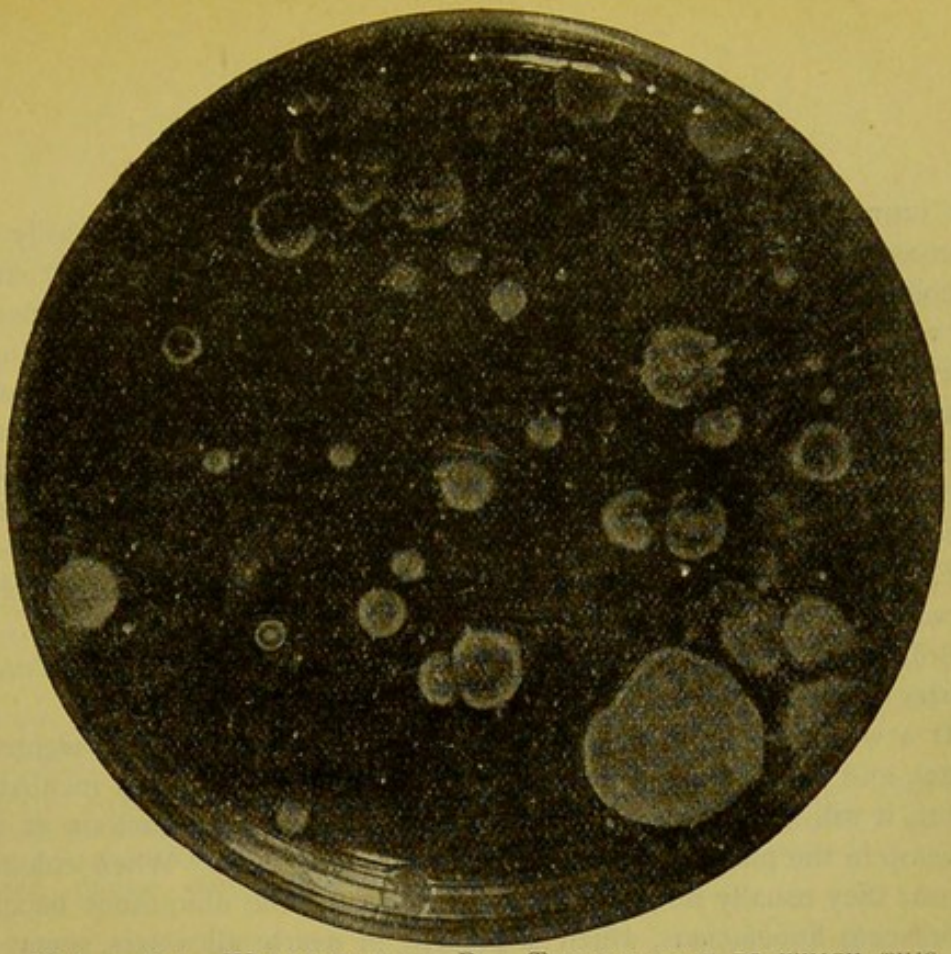
* The apparent absence of increase of the non-liquefying colonies was evidently due to the greater number of liquefying colonies. The equality of the numbers on the third and fifth days was certainly the result of an accidental coincidence.

B. Water (B_{37}) *collected on November 30th*, 1897.

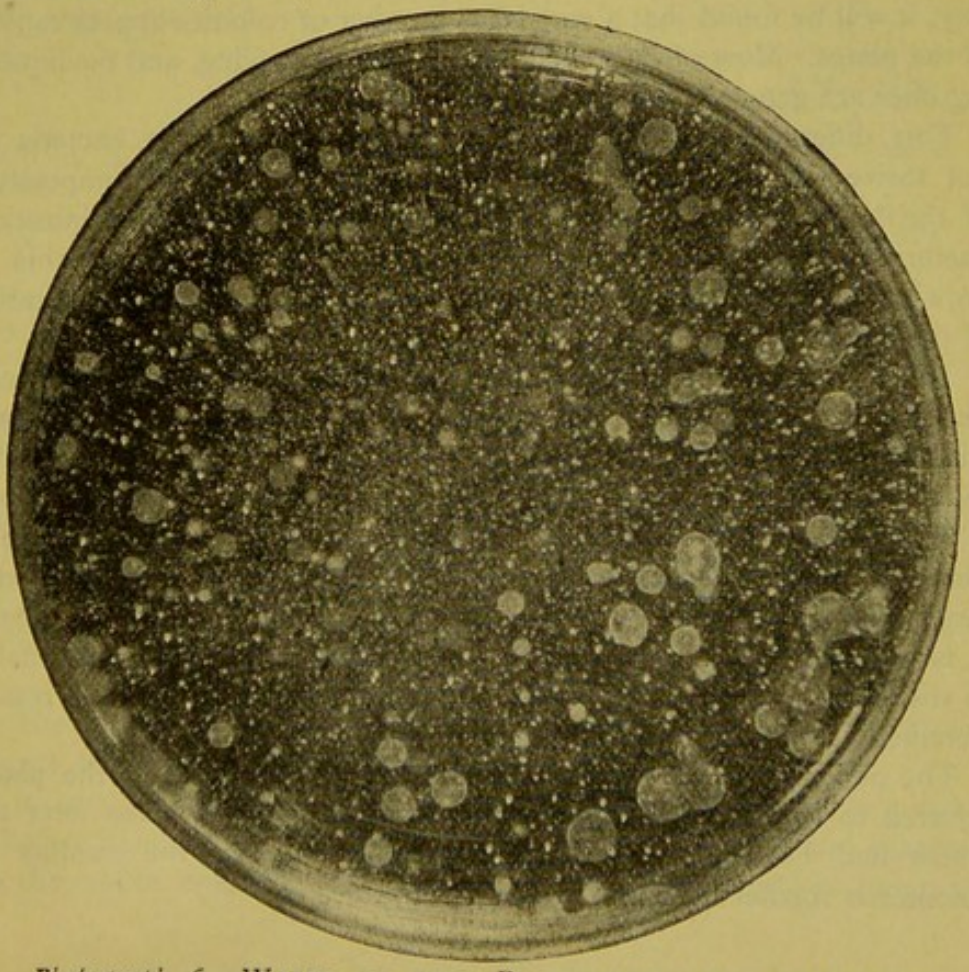
	⅛ gramme.			⅛ gramme.		
	Liquefying.	Non-liquefying.	Total.	Liquefying.	Non-liquefying.	Total.
2nd day	—	121	121	—	154	154
3rd day	22	253	275	—	—	—
5th day	33	275	308	44	308	352
6th day	No further notable increase.					

I have selected these special instances because, owing to the moderate number of colonies, it was possible to count them all on each day, so that the numbers given are absolutely reliable.

I have already insisted upon the importance of not relying on fractional countings, that is, when the number of colonies is sufficiently small to allow the whole plate to be counted. I should have said also that the best contrivance to facilitate the enumeration of colonies is that known as Wohlfiigel's apparatus, the essential part of which is a glass plate ruled into squares of 1 c.m. side. It is sometimes recommended for counting colonies in Petri dishes that a circle divided into sectors of equal size be used. Even when the whole plate is counted this contrivance is unsatisfactory. It is very difficult *near the centre* of the circle to see what colonies belong to each sector, and when the colonies



Photograph 5.—Water of well B_9. The colliery to which this well belonged was worked at the time. Water not incubated. ($\frac{1}{2}$ gram.)



Photograph 6.—Water of well B_9 incubated for seventy-two hours. ($\frac{1}{4}$ gram.)

are numerous, accurate counting is very difficult. This can easily be overcome by including the central parts in a central circle, but such a counting plate would present no great advantage over Wohlffügel's or any similar arrangement. When a microscope with a large stage is available, much time may be saved by counting the colonies under a magnifying power of fifteen to twenty diameters, with which it is easy to recognise colonies which are not distinct when the usual magnifying glass is used. This also allows their characters to be clearly made out at the same time.

E. The differences observed in the rate of multiplication of bacteria in waters of various qualities have already been pointed out.

Still more remarkable differences may be brought out by *incubating waters at the temperature of the body*.

If a water free from pollution be incubated at 37° for about eighteen hours, and then plates prepared in the usual way with this incubated water, it will be found that very few, and sometimes no bacteria at all, develop in the plate in the course of four or five days. When colonies appear they usually do not liquefy the gelatine; the ubiquitous bacillus fluorescens liquefaciens, which is present in nearly all waters, seems to disappear entirely.

If, on the other hand, water fæcally polluted be incubated in the same way, it will be found that a very large number of colonies appear rapidly in the plates. Most of these colonies are non-liquefying, and the liquefying ones are generally small and non-chromogenic.

This difference is due to the fact that ordinary water bacteria do not thrive well in media rich in animal proteids at the temperature of the body, and that their growth is even inhibited. The intestinal bacteria found in polluted water are, on the contrary, capable of growing and multiplying rapidly in water kept at the temperature of the body.

Table VI. seems to show that incubation of waters of doubtful purity may be of use for the purpose of detecting pollution. I have tried this method in a number of instances in which the presence or absence of pollution were practically certain, and I have found it to give unexpected results in one case only. In that case I found a water, which was apparently good, yield after incubation a large number of bacteria, but, as the water came from an old main, it is possible that the method was not at fault, although I was unable to discover a cause of pollution capable to account for the results obtained.

The photographs 1 to 6 give a pretty fair idea of some of the plates referred to in Table VI. These photographs were all taken after the plates had been incubated two days only, so that the number of colonies is smaller than that given in the table.

TABLE VI.

Source of Samples.	Water not incubated.				Water incubated at 37° C. A for 18 hours.			
	Day of final counting.	Bacteria in 1 gramme.			Day of final counting.	Bacteria in 1 gramme.		
		Liquefying.	Non-liquefying.	Total.		Liquefying.	Non-liquefying.	Total.
B_1. Uncontaminated surface spring.	7th	2	41	43	4th	—	—	—
B_3. Brooklet unpolluted, but accessible to poultry (and occasionally to cattle).	4th	16	60	76	4th	—	58	58
B_2. Stream receiving B_1 and B_3, *plus* the drainage of a farm.	7th	33	425	458	4th	—?	3,225	3,225
B_4. Stream exposed to occasional pollution during rainy weather; water collected during fine weather.	4th	8	75	83	4th	8 small white	—?	8
B_7. Reservoir receiving polluted and good feeders; water collected when the weather was fine and the reservoir undisturbed.	6th	33	117	150	4th	—	—	—
					B for 72 hours.			
B_9. Well in a colliery (worked).	3rd	216	3,800	4,016	2nd	numerous small white colonies	numerous small white colonies	47,520+
B_{17}. Spring (possibly contaminated).	4th	8	183	191	3rd	—	1	1
B_{16}. Same water as B_{17} after it has received the drainage of a farm.	2nd	733+	7,000+	7,733+	2nd	innumerable	innumerable	∞
B_{14}. Stream receiving *constantly* a small amount of fluid manure.	2nd	58+	141+	141+	3rd	10	626,400	626,410
B_{18}. Reservoir receiving B_{14}, B_{16}, B_{17}, water collected on a fine day when the reservoir was undisturbed.	4th	16	75	91	5th 11th	—? 30	3,000 3,000+	3,030+

The effects of incubation at the temperature of the body may also be shown more rapidly and almost as clearly in another way.

A small quantity of water, 1 c.c. for instance, is added to 5 c.c. of alkaline bouillon. This mixture is incubated at 37° C., and examined at the end of one, two, and three days. It will generally be found that

at the end of forty-eight hours the bouillon containing only water bacteria will have remained clear, or nearly so. The bouillon to which fæcally polluted water has been added will, on the contrary, be found quite turbid and generally covered with a thick bacterial scum or pellicle. This is well shown in photograph 10, to which I will have to allude again.

6. *Practical application or investigation of a water supply.*

I have now to show how the methods previously described may be applied in practice and how, notwithstanding the limitations I have indicated, they can give assistance in demonstrating the existence, or locating sources, of pollution.

I will select a few examples only for purposes of illustration. These occurred in the course of two investigations which I carried out during the last and the present year.

In these two investigations, which lasted several months, I was assisted by my friend Dr. E. J. Sidebotham, and I consider myself fortunate to have had the co-operation of so able a colleague. All the examinations were conducted strictly according to the lines laid down in this paper.

A. *Survey of a water supply derived chiefly from four upland gathering-grounds.*

The water was unfiltered. The object of the investigation was (1) to discover whether the gathering-grounds were dangerously polluted; (2) if polluted, whether the water, after passing through the reservoirs and reaching the consumer, still retained traces of pollution; (3) whether removal of the sources of pollution was practicable and would secure a good water supply.

As my object is simply to give practical examples of the applications of bacteriological examinations I will not describe the gathering-grounds, nor give a complete account of all the analyses made.* The examples I have selected are tabulated in the chart appended to this paper.

The first thing was to obtain *samples of water from unpolluted feeders* such as streams, springs, or land drains situated in unpolluted parts of the watersheds. Four of those feeders, which may be designated by the numbers in the chart, gave the following results:—

Feeder 2 at a point 250 yards from the impounding reservoir, and 407 yards from the nearest upper limit of the watershed, running over a stony bed through uncultivated moorland, was found to contain 34 bacteria to the gramme during fine weather. Water from a similar stream (2), but running over a softer bed and passing through some patches of peat, was collected on a rainy and windy day after several days' heavy rain; on that occasion it contained 210 colonies. These

* All this will be found in the full report when published.

two streams, being closely comparable with regard to possible chances of pollution, have been placed together in the chart.

Feeder 3 at a spot 100 or 150 yards above the nearest inhabited house, 418 yards from the reservoir, and 810 yards from the nearest part of the upper limit of the watershed, contained 57 bacteria to the gramme during fine weather.

Feeder 4 was examined at a place 1780 yards from its entrance into the reservoir, 200 yards from the nearest upper limit of the gathering ground, and about 50 yards above the nearest farm. Fowls had free access to the banks of the stream, which, possibly, was also at times visited by cattle. The number of bacteria found during fine weather was 76 (71 is given in the chart by mistake).

Two land drains (5), used for collecting the water from some pasture land to which cattle had free access, were also examined. In one of them, during fine weather, the water was found to contain 41 bacteria; water from the other was collected during heavy rain, and after several days' almost continuous stormy weather it contained 280 bacteria; yet the soil over the latter was partly covered with cow dung. This land drain was specially selected in order to ascertain whether water gathered by means of land drains running under cattle pastures was seriously polluted by the excrements deposited on the surface. In this case a layer of eighteen inches of soil (including the turf) was apparently sufficient to intercept all, or nearly all, the bacteria, with which the superficial layers of the ground were teeming. After removing a sod I collected some of the soil exactly over the drain (about 16 inches above it and $1\frac{1}{2}$ inch below the actual surface of the ground). A known quantity of that soil was mixed with a known quantity of sterilised water. After thorough shaking, a quantity of water corresponding to $\frac{1}{200}$ gramme of soil was used for preparing plates. After incubation for two days only, the plates indicated the presence of at least 42200 bacteria in each gramme of soil. Owing to the rapid liquefaction of the plate it was impossible to count the bacteria again, but it is safe to assume that the numbers found represented only a fraction of the total number of bacteria which must have been present in that soil. Yet the water running sixteen inches deeper did not contain more than 280 bacteria, and that at a time when the ground was soaked with water. We have just seen that during fine weather the water of a similar drain contained only 41 bacteria.*

* To test whether water passed actually from the superficial layers of the soil into the drains at the place under investigation, I took samples of the surface soil and of the water of the drain for chemical analysis. It was found by Professor Dixon, to whom I sent these samples, that the contaminated soil contained about twice as much chlorine as the uncontaminated soil. The water of the drain passing under the polluted soil contained from 1·5 to 2 parts per million more chlorine than the water of land drains passing under unpolluted areas. This increase in the chlorine indicates that soluble salts were able to pass from the surface into the drains.

For purposes of comparison I took on the same day, exactly in the same way, some soil free from any pollution from the banks of a small uncontaminated feeder, and found that only 24600 bacteria had grown by the end of the second day. Comparatively few liquefying colonies developed; the total number of liquefying colonies found in 1 gramme, on the fourth day, was only 400. The number of bacteria present in the water of the stream was by a strange coincidence found to be 280, that is to say, the same number as that found in the drain under same conditions of weather. I need not say that this similarity of numbers was purely accidental.

From these observations it may be concluded that uncontaminated streams in these special gathering-grounds might contain during fine weather up to 70 and possibly 80 bacteria, and during rainy weather up to 210 or 280 bacteria to the cubic centimetre; 70 could therefore safely be considered as the highest limit of bacterial impurity during fine weather, and 280 the highest number during rainy weather in this particular case.

These two numbers gave us special standards for the grounds under investigation, the dry-weather standard indicating what may be called the *permanent bacterial impurity*, the wet-weather standard indicating what may be described as *fluctuating bacterial impurity*.

I do not wish to claim an absolute rigidity for those standards, but I think they constitute a better basis of comparison than the arbitrary standards of 100, 500, 1,000, given by various observers without any reference to the state of the weather. With regard to water filtered through sand, it seems to me that 100 is much too high a number to allow, considering that unfiltered water running in open streams contains, when unpolluted, much less than 100 bacteria. No disturbance should be produced by rain in sand filter beds.

I will now take the case of a *feeder continuously polluted* by very foul drainage from a farm. This farm was situated about 430 yards from the nearest upper limit of the gathering-ground and 500 yards from the reservoir. Pigs, fowls, and ducks were kept. The pail system was used, and the ground round the farm was manured with the night soil.

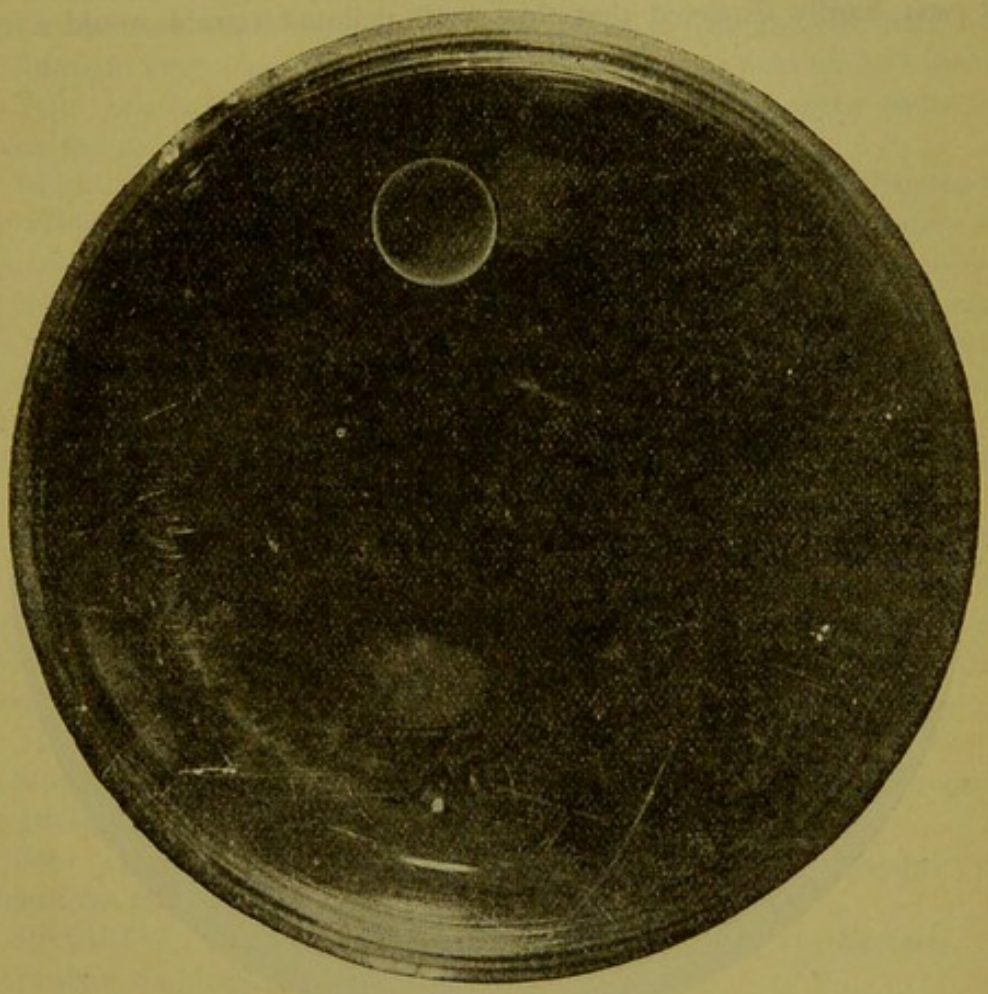
Drains from the farm opened directly into the feeder under investigation, which was only a small brook during dry weather.

About 240 yards above this farm a surface spring emerged from the side of the hill. Its water was collected in an iron tank, from which it passed again underground into drains, which after running through the farm reached the brook above mentioned.

Samples of water were taken on a fine day from the spring above the farm, and, a few minutes after, from the brook, about 50 yards below the farm. Shortly after, a third sample was taken from the reservoir

itself at a distance of about 100 yards from the opening of the polluted feeder. The results of these examinations were as follows :—

The spring above the farm (8 in the chart) was found to contain 191 bacteria. This was unexpected, and probably due to some deposit of manure which escaped our notice. This suspicion was confirmed by



Photograph 7.—PLATE PREPARED WITH ½ GRAM OF THE WATER OF THE SPRING 8 ABOVE THE FARM AFTER TWO DAYS' INCUBATION AT 21°—22° C. NOTE THE ABSENCE OF LARGE WHITE, OPAQUE LIQUEFYING COLONIES OF PUTREFACTIVE BACTERIA.

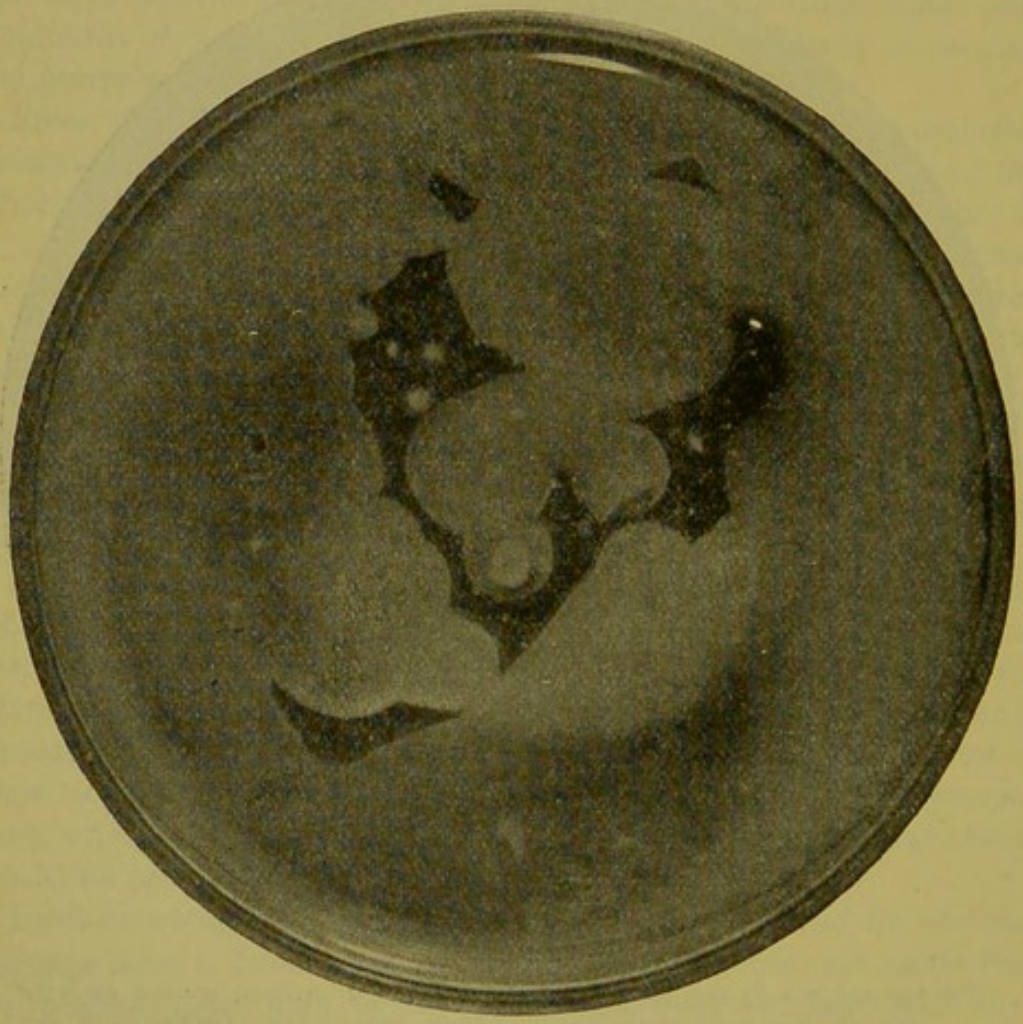
Professor Dixon's chemical analysis, which revealed a distinct (though slight) excess of chlorine, the water being in other respects chemically good.

After incubation at 37° for 72 hours this water yielded only ten non-liquefying colonies, so that, on the whole, it could not be said to be badly polluted.

The stream below the farm (5 in the chart) after receiving the drainage was found to contain an immense number of bacteria. On the third day of incubation the plates were found to contain more than

7733 bacteria, and, owing to rapid liquefactions setting in, it became impossible to count even the plates prepared with $\frac{1}{10}$ gramme of water. After incubation at 37° for 72 hours, the number of colonies, both liquefying and non-liquefying, was so large that counting was impossible.

The reservoir (18 in the chart) received other very good feeders, and it was hardly expected that this small polluted stream would affect



Photograph 8.—Plate prepared with ½ gram of water of the polluted stream 15 below the farm after two days' incubation at 21°—22° C. Note the large number of opaque liquefying colonies.

considerably the purity of the water. The number of bacteria during fine weather was 91, and during wet weather 420. These numbers were distinctly higher than those obtained in connection with pure feeders. After incubation of the water for 72 hours at 37°, 3000 non-liquefying and 30 liquefying colonies were obtained, this showing clearly the presence of objectionable bacteria. The same conclusion was also indicated by the appearance of the plates prepared in the usual way, three of which are shown in photographs 7, 8, and 9.

Another case to which I have already alluded at the beginning of this paper shows clearly the use of reliable standards.

A stream forming the chief feeder of a reservoir was indicated to me as a feeder of undoubted purity; the officials knew of no source of pollution; the stream ran over a stony bed, through what was thought to be uncultivated and uninhabited land. A first sample was taken in November, the weather being fine, and found to contain 141 bacteria. A fortnight after a sample was collected during rainy weather and found to hold 460 bacteria. I came to the conclusion that there must be some source of pollution on that stream.

In order to test the truth of this conclusion I asked Dr. Sidebotham to follow the stream from its entrance into the reservoir towards its source, taking samples of water at various points and looking for possible sources of pollution. About 250 yards above the mouth of the stream he found that one of the banks was covered with manure, part of which seemed to be of human origin, and in addition there was a superficial drain discharging slowly into the brook fluid manure, oozing from a large heap of stable manure situated more than 150 yards from one of the banks of the stream.

The reason why these things had escaped notice was that, in its course from the higher ground to the reservoir, the stream fell from a height down a high, almost vertical, rocky wall, which hid from view all the upper parts of the ground to those visiting the more accessible lower regions.

The samples collected on that day yielded the following results :—

Stream (30), 150 yards above the fall (420 yards from reservoir), 34 bacteria, mostly of two kinds.

Pool (31), on the course of the polluting drain, 17500+ bacteria, of more than ten kinds.

Stream (32), at the fall (250 yards from reservoir), 155 bacteria, of more than six kinds.

I will now show how the *intermittent action of distant sources of pollution may be detected*.

A pretty large stream (6) was found to yield, during dry weather, 83 bacteria. The only inhabited house above the place where the sample giving this result had been taken was a farm which did not send any drainage directly into the stream. At the point where the stream came nearest to this farm the ground was undulated, and there was no *direct* fall from the house to the water. At that place the stream was 220 yards from the farm. It was therefore doubtful whether the small excess of bacteria found during fine weather meant anything.

I therefore investigated this stream again during rainy weather.

Beginning near its source, about one mile from the reservoir and 530 yards above the suspected farm, I took samples of the water of this

feeder and of one of its tributaries. Within half an hour I took other specimens from the same stream, at the place where the first samples had been taken during fine weather, *i.e.*, about 460 yards from the farm.

The following results were obtained :—

Feeder (2) above the farm (rainy weather)	210	bacteria.
Tributary of feeder (4) (rainy weather)	280	,,
Feeder (6) below the farm (rainy weather).....	2055+	,,
,, ,, ,, ,, (dry weather)	83	,,

It was quite evident from these results that during fine weather the soil round the farm could absorb and retain all, or nearly all, the drainage or manure deposited round the house, but that the case was different during heavy rains. I must add that the soil in that part was not at all retentive and was easily waterlogged.

I think it unnecessary to give any further instances of the results which may be obtained by systematic bacteriological surveys. In the chart some of the results obtained during the investigations I have just referred to are arranged in such a way as to indicate what may be expected from such investigations.

I would point specially to group L, which shows the effects of the pollution of a feeder, and the gradual purification which takes place (chiefly by sedimentation) in the reservoirs and mains. The improvement taking place in the mains has, I think, not attracted sufficient attention. Whenever the water of mains contains more bacteria than, or as many as, the water of the corresponding reservoir, at a distance of one mile or more from the reservoir, this should suggest some defect.

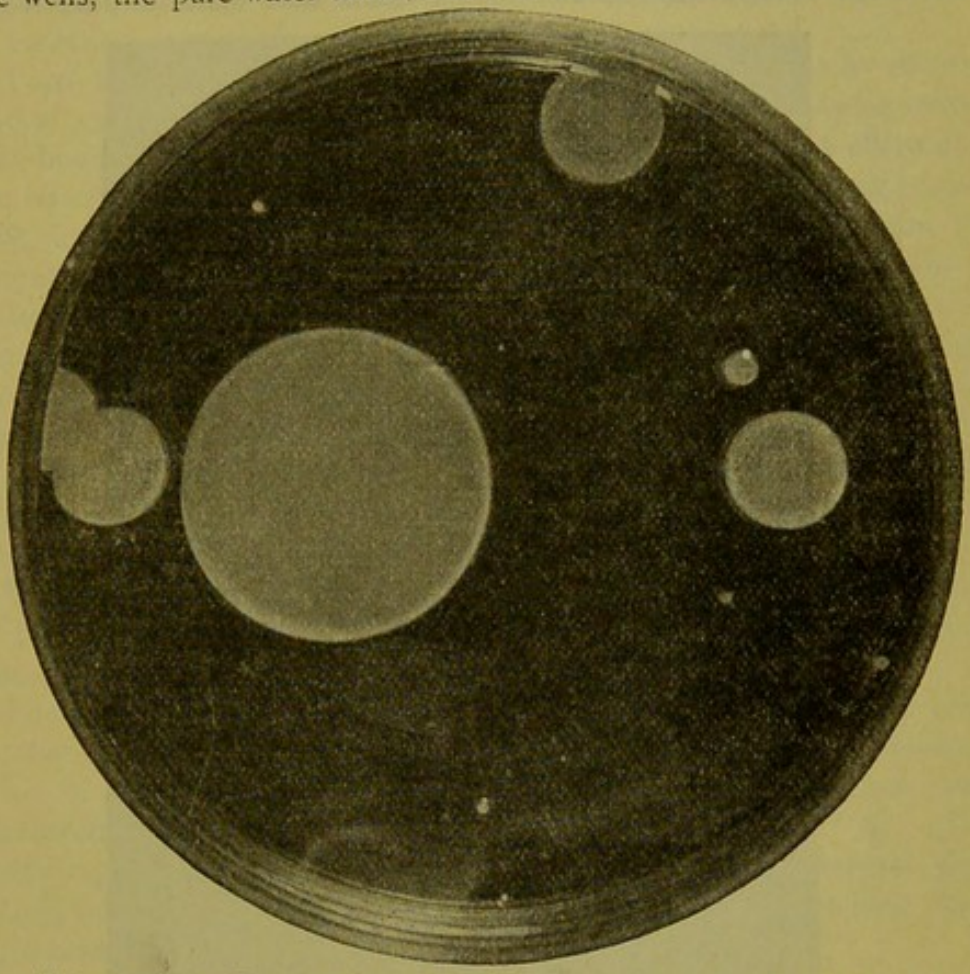
Though it is not my intention to deal with filtration of water, the following observations are so clearly connected with the subject of this paper that I think they are not out of place here.

Two reservoirs, which I will call A and B, were situated in the same gathering-grounds at a distance of about 1200 feet from each other; one of them (B) was about 150 feet higher than the other. The upper reservoir (B) received good water from the upper unpolluted part of the catchment area. The lower reservoir (A) was badly polluted. The water of both reservoirs was filtered through sand before being distributed to the town.

The filters were small and apparently well constructed; the layer of fine sand was more than 3 feet deep. At the time when they were examined the rate of filtration did not exceed $1\frac{1}{3}$ gallon per square foot per hour; the pressure was not more than 20 inches of water. One had therefore to deal here with a very interesting combination of circumstances.

This investigation was started owing to the results of a bacteriological examination of the water distributed by the two mains respectively connected with the reservoirs above mentioned.

In both these mains the water contained on one occasion more than 300 bacteria. Samples were subsequently taken from the two reservoirs, the wells, the pure-water tanks, and the mains connected with the filter



Photograph 9.—PLATE PREPARED WITH ½ GRAM OF WATER FROM THE RESERVOIR 18 AFTER INCUBATION FOR TWO DAYS AT 21°—22° C. NOTE THE PRESENCE OF LARGE LIQUEFYING OPAQUE COLONIES ABSENT IN PLATE 7 AND ABUNDANT IN PLATE 8. THESE BACTERIA, MOST PROBABLY, CAME FROM THE POLLUTING DRAINS.

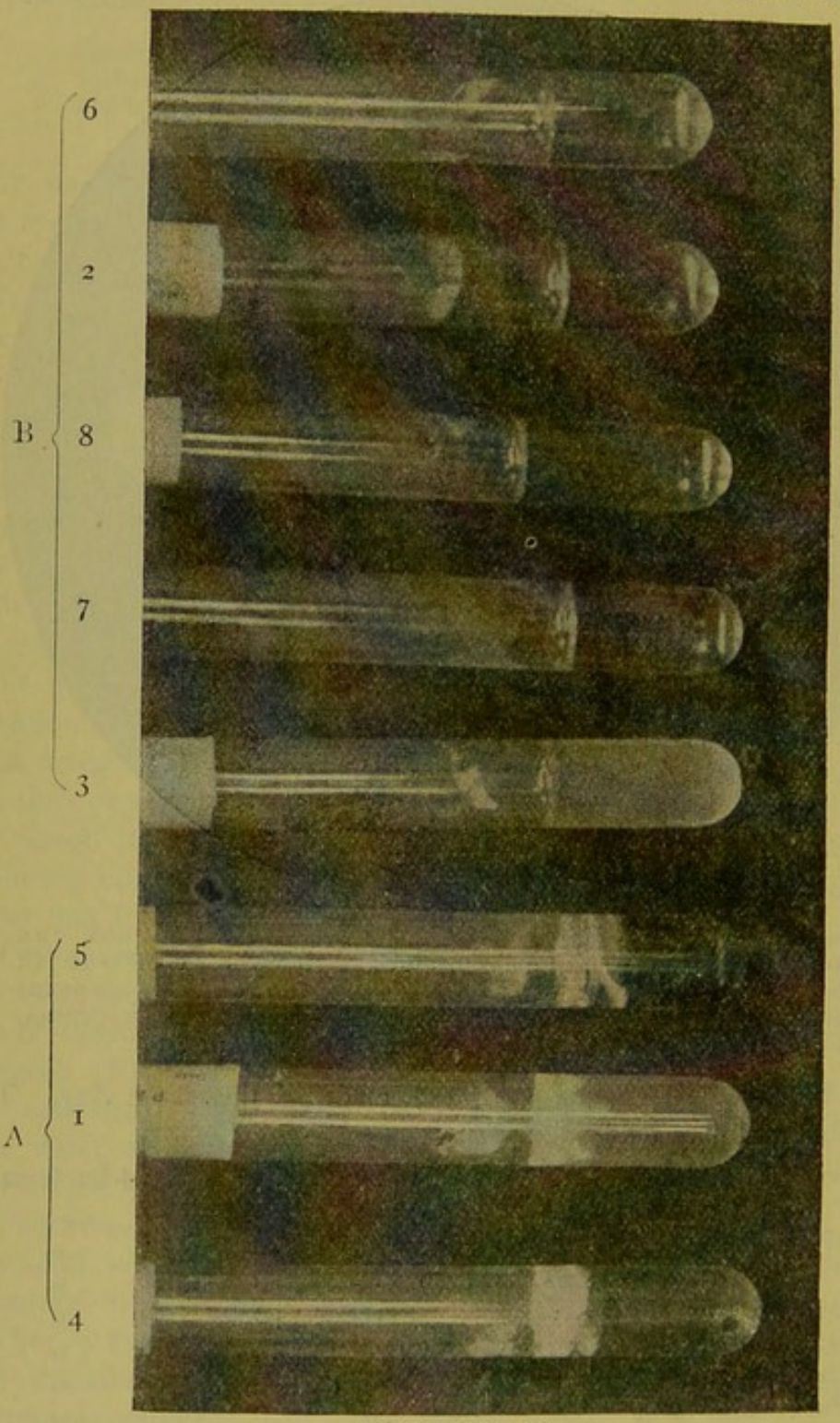
beds. All these specimens were collected on the same day. The results obtained may be tabulated as follows:—

Reservoir A, polluted	129+	bacteria.
Pure-water tank	67	,,
Hydrant 2¾ miles from reservoir	51	,,
,, on a previous occasion	334	,,
Reservoir B, unpolluted	39	,,
Outlet of filter No. 1 (worked 1 month) ...	868	,,
Outlet of filter No. 2 (worked 3 days) ...	920	,,
Pure-water tank	1078	,,
Hydrant 2½ miles from reservoir	3164	,, *
,, another time	356	,, †

* Water had not been allowed to run sufficiently long.
† Water allowed to run about half an hour.

It was evident that filter B, instead of purifying the water, added to it a large amount of bacterial impurity.

In order to find the cause of this, I had a hole made in filter No. 1



Photograph 10.

through the sand and subjacent layers of gravel and broken stones, and finding nothing to account for the deterioration of the water, I had the drains under the filter bed exposed. There a layer of slime com-

posed almost entirely of bacteria was found covering part of the silt which had accumulated in the drains. Samples of the turbid water collected in three of these drains gave respectively the following number of bacteria : 4100, 2450, 40350 !

I also tested the water of the reservoirs, filters, and mains by incubation at 37°. The water was incubated in test tubes after being mixed with bouillon. Two days after, it was found that the tubes containing the water from reservoir A, whether filtered or unfiltered, were all turbid. The cloudy fluid was also covered with a thick white pellicle. On the contrary, the water of reservoir B, notwithstanding the large number of water bacteria it contained, had remained absolutely clear in all cases but one, in which the fluid became slightly turbid ; this turbid tube had been prepared with water that had passed through one of the filter beds.

In the first case the filter, though effecting a certain amount of purification, *must have allowed the passage of a number of putrefactive if not pathogenic bacteria.*

In the second case, the filter, though causing undoubted deterioration of the water, *had been safe only because the water was good before it was filtered.*

Photograph 10 shows the appearance presented by the tubes after incubation for more than two days. Each one of these tubes contained 1 c.c. of water mixed with 5 c.c. of alkaline bouillon and had been incubated for forty-eight hours at the temperature of 37° C. (The photographs were taken after the tubes had been kept several days longer, but no important change had taken place after the first forty-eight hours.)

A. Polluted reservoir.
4. Reservoir water.
1. Water from main $2\frac{3}{4}$ miles from reservoir.
5. Water from pure-water tank.

B. Unpolluted reservoir.
6. Water from reservoir.
2. Water from main $2\frac{1}{2}$ miles from reservoir.
3. Pure-water well of filter which had worked one month.
7. Pure-water tank receiving 3 and 8.
8. Pure-water well of filter which had worked for three days only.

I need not say that such an experience emphasises *the danger of relying upon filtration as a safeguard against the effects of pollution.* We have had numerous instances showing that filtration has failed in times of need. Sand filtration to be effective must be conducted with a considerable amount of care and watchfulness, and is liable to accidental breakdowns, which it is not always easy to anticipate. When gathering-grounds are so situated that they can be protected against pollution,

protection alone is *better than filtration alone*, and filtration should never be taken as an equivalent to protection.

By this I do not mean to say that filtration is useless; on the contrary, I think that whenever filtration can be added to protection of gathering-grounds this should be done, provided it does not serve as *an excuse for allowing the access of filth to our water supplies*. I think the results recorded above show clearly that surface water, collected on high moorlands and not polluted, is very pure, and certainly better than polluted waters may be after sand filtration. The objection to these waters being used unfiltered is due to the presence of suspended organic matter which may become abundant during stormy, rainy weather. This matter can of course be removed by filtration, but other processes acting mechanically, such as properly conducted sedimentation and straining, should answer the same purpose in a simpler way and with less need of expert control. It must not be forgotten also that, in order to secure absolutely clear water by filtration, filter beds should be provided *for each service reservoir*, or else these reservoirs must be constructed with great care, covered, and adequately protected from all sources of pollution. Unless adequate protection be ensured, filtration of water above these reservoirs will give a false security, and not prevent the turbidity which may arise from disturbances in the service reservoirs.

LONDON:
Printed by Jas. Truscott & Son,
Suffolk Lane, E.C.