CHAPTER XI.

ANALYSIS AND INTERPRETATION OF RESULTS.

The results of a bacteriological or chemical analysis of a sample of water are necessarily expressed numerically and in a technical way. It is possible, however, without discussing the details of the various processes used by chemists and bacteriologists, to understand the figures and the deductions which may be drawn from them. The minute proportions in which some of the most significant impurities exist in drinking waters render the analysis exceedingly difficult and delicate. The difference between a pure and an impure water may only be indicated by a few parts in 100,000; and the problem is further complicated by the fact that, as animal and vegetable substances contain practically the same elements, it is often difficult for the chemist to decide whether the pollution is of animal or vegetable origin. As the quantities are so small, it is very rarely that their exact nature can be ascertained, so that usually the decomposition products only are determined. There is no chemical test sufficiently delicate to indicate with certainty whether an organic impurity in a natural water be poisonous or innocuous. On the other hand, the information furnished by an analysis

gives valuable suggestions as to the quality of a water, especially if its source be known and the data of its normal composition have been previously ascertained.

The results of an analysis are still commonly expressed in grains per gallon of water, *i.e.*, in parts per 70,000. The method of stating the results in parts per 100,000 is, however, far preferable, inasmuch as being founded on a decimal system, they are at once comparable with analyses made in other countries. Continental results are sometimes stated in grammes per litre (parts per 1,000), whilst occasionally parts per 1,000,000 (milligrammes per litre), have been adopted. Results expressed as grains per gallon can be converted into parts per 100,000 by dividing by seven and multiplying by ten, whilst multiplying by seven and dividing by ten converts parts per 100,000 into grains per gallon. A Committee of the British Association recommended that all water analysis results should be expressed in parts per 100,000, and many authorities have since adopted that plan, which is the one used in this book.

Samples of water for analysis should be taken in the stoppered half-gallon bottles known as "Winchester quarts," which are obtainable at most chemists. They should be free from any adhering dirt and washed out with concentrated sulphuric acid when purchased, then filled up with common water and rinsed several times, finally with distilled water.

In collecting the sample the precautions mentioned

on p. 17 should be observed. The bottle should be filled to the top with the water, then rinsed out with it, filled, and the stopper rinsed and inserted. Except when the gases dissolved in the water are to be examined, it is best to leave a small air space below the stopper. If possible the temperature of the water should be observed at the time of collecting the sample. Any surrounding circumstances—distance of dwelling, &c., nature of soil, depth of well, presence of plants, &c., —should be noted. After the sample is collected it should be despatched as quickly as possible to the analyst, as many waters change very considerably on keeping.

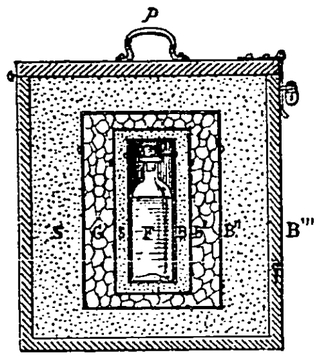

FIG. 49.—Ice Case for Bacteriological Samples.

Samples required for bacteriological examination should be separately taken in sterilised bottles, about two ounces in capacity, and immediately packed in ice (Fig. 49) and forwarded for examination. For an ordinary chemical analysis one Winchester quart of the water is sufficient, but when a mineral analysis is required two or three times this amount will be found necessary.

The interpretation of results of analysis is often a matter of considerable difficulty, as the analyst judges of the purity or otherwise of a water upon all the

factors presented to him, and not on any single constituent. Some authorities insist upon withholding from the analyst particulars as to the source and possible contaminating influences of a water sent for analysis, thinking that by so doing his opinion will not be biassed in any way. Such procedure is, however, most undesirable, as it must obviously be to the interest of the senders to arrive at the truth, and any circumstances which may give rise to suspicion may be very helpful to the analyst as explaining some of the figures which he may obtain, which otherwise he might consider not sufficiently condemnatory to warrant his pronouncing against the supply.

The deductions to be drawn from the general appearance, colour, and odour of a water have already been mentioned in Chapter I.

The total solids are obtained by carefully evaporating a measured volume of the water, drying the residue at 120° C., and weighing it. The solids in a good drinking water should not amount to more than thirty or forty parts per 100,000, and should be white and crystalline, or finely granular, and not coloured in any way. Frequently a water sample contains matter in suspension, and it becomes a question whether the suspended matter should be included in the total solids or separately recorded. As a water sample is taken usually by inexperienced persons, it is exceedingly unlikely that the suspended matter collected in

a Winchester quart represents fairly the average amount of matter in suspension in the water, so that, in most cases, the analyst prefers to separately estimate this amount. The total solids are therefore determined upon a sample of the water taken from the bottle after it has been allowed to stand for some hours, when the grosser particles will have subsided to the bottom of the bottle.

The loss on ignition represents the amount of loss which the total solids undergo when the dish containing them is heated to low redness. If there is much organic matter present the solids blacken under this treatment, and if this organic matter is of animal origin an odour of burnt feathers, indicating the presence of much nitrogenous matter, is noticed. The ash may be coloured brown if iron is present in the water, but is usually white, and consists of the mineral salts present. Many mineral salts, *e.g.*, magnesium chloride, lose acid on being heated in this way, so that the loss on ignition is not an absolute measure of the amount of organic matter present in a water. To overcome this difficulty some analysts add a known amount of sodium carbonate to the solid residue before igniting, in order to fix any such acids which might otherwise be evolved.

The total amount of chlorine as chlorides, as already mentioned, is determined volumetrically by a standard silver solution. The result is returned as chlorine, and also in terms of sodium chloride. It must not be

forgotten, however, that many waters naturally contain other chlorides, so that the assumption that the whole of the chlorine is present as sodium chloride is not always warranted. A high chlorine, however, usually raises a suspicion of contamination with sewage, as urine contains about 1 per cent. of sodium chloride, About 1·5 to 3·0 parts of chlorine per 100,000 is a normal amount; but in districts where there are salt deposits, as in Cheshire, or in wells in the New Red Sandstone or in proximity to the sea, the water may normally contain a higher amount without indicating sewage pollution. In the United States the influence of the sea on land water has been carefully studied, and Dr. Drown, in his reports to the Massachusetts State Board of Health, has shown that it is possible to map out the State by lines which are practically parallel to the coast line, in which the ground water shows equal amounts of chlorine. Such lines he terms "isochlors," and in his hands they have proved of considerable value, as any excess of chlorine found in any well water above the natural "isochlor" shows at once local contamination.

The amount of chlorine found in a water can be converted into its equivalent amount of sodium chloride, NaCl, by multiplying by the factor 1·65. Although chlorine as chlorides thus gives a measure of the amount of sewage pollution that the water has received, it does not give any information as to when such pollution took place, since, by filtration and

oxidation, the organic matter of the sewage and the pathogenic organisms possibly present may have long since been entirely removed from the water.

By the term *oxygen consumed* by the organic matter in a water is meant the reduction which an acidified solution of permanganate of potassium undergoes when brought into contact with a known volume of the water. This test is conducted in various ways, and different analysts use solutions of permanganate of different strengths, and allow it to act on the water under various conditions of time and temperature. The red colour of the solution is gradually destroyed, very polluted waters removing the colour almost instantaneously. By using a solution of permanganate of ascertained strength, the amount of reduction is determined by adding excess of potassium iodide, and titrating with a standard solution of thiosulphate. The method most commonly followed in this country is to determine the amount of oxygen consumed at 80° F. in two stages :—

1. *In fifteen minutes:* this figure includes the nitrites and any ferrous salts, sulphides, and any very easily reduced organic matter.

2. *In four hours:* after this time the whole of the organic matter will have been oxidised from most waters, but with very bad waters a longer time is still required to finish the oxidation. In Germany it is customary to boil the water with the acidified permanganate for one hour; whilst the author is in

the habit of keeping the water and permanganate for three hours in a stoppered bottle at a temperature a little short of boiling, so as to get a maximum amount of reduction.

Attempts have been made to calculate the relation between the amount of oxygen required and the amount of carbon present in the water as found by combustion, but no definite relation seems to exist, since the factor varies with waters of different characteristics. Where, however, consecutive determinations have to be made on the same supply, the oxygen absorbed approximately represents the carbonaceous matter, and varies, like the albuminoid ammonia and the chlorides, with the fluctuations of the seasons, so that any abnormal deviation at once points to some new source of pollution.

The condition in which *the nitrogen* derived from animal organic matter exists in a water is one of the chief points which a full chemical analysis determines. A water contaminated with sewage will contain a definite amount of chlorides and nearly all nitrogenous matter with which such chlorides were originally associated. If, after pollution, the water has been under the influence of bacteriological action, the nitrogen may have been converted into oxidised forms; and, therefore, in most cases a water contains nitrogen in the several forms of organic compounds, ammonia, nitrites, and nitrates. Fresh sewage is practically free from nitrates, whilst a deep well, or

well-oxidised river water, contains the nitrogen almost entirely in the form of nitrate. The ratio of the oxidised to unoxidised nitrogen in a water, therefore, gives a measure of the amount of purification which has taken place, and the total nitrogen of all kinds the absolute amount of pollution which the water has sustained. Under certain conditions, however, some of the nitrogenous compounds are so completely destroyed by bacterial agencies that nitrogen gas and the lower oxides of nitrogen are evolved, and a loss of total nitrogen is therefore caused. When the quantities of nitrogen in a water are compared with the amount of chlorine, it is found that the chlorine is largely in excess, although in urine the amount of nitrogen is slightly greater than the amount of chlorine. This difference between theory and the amount actually found is due to the absorption of nitrates by plants, and only in raw sewage do we find that the amount of nitrogen at all approaches the amount of chlorine.

The term *albuminoid ammonia* is given to the quantity of ammonia which can be obtained from a water after the removal of the saline, or free ammonia, when such water is boiled with an alkaline solution of permanganate. The process was first devised by Wanklyn and Chapman, who showed that, although the total organic nitrogen was not obtained in this way in the form of ammonia, all polluted waters gave off a fraction of the nitrogen in this form, so that

the relative amounts of albuminoid ammonia fairly represent the amounts of unoxidised organic or polluting matter actually present. Before determining the albuminoid ammonia, it is necessary to remove the free ammonia, so that a determination of the amount of free ammonia is first made.

Free ammonia and albuminoid ammonia.—For this determination about half a litre of the water, made alkaline with carbonate of soda, is distilled until the free ammonia has passed over, and the amount estimated by the brown colour given by Nessler's reagent. To the remainder in the retort a solution of potash and potassium permanganate is added, and the distillation continued until the "albuminoid ammonia" has all come over; the amount is estimated by means of Nessler's solution, as in the case of the free ammonia. A large quantity of free ammonia is generally indicative of recent sewage contamination, as it is frequently formed directly from urea by bacteria. Vegetable matter gives rise to little or no ammonia on decomposition.

As already mentioned, the albuminoid ammonia is only a relative quantity, and does not give the absolute amount of organic nitrogen present in a water. In many of the recorded cases of water-borne typhoid the amount of albuminoid ammonia found in the water was so extremely small that the supplies would seem from the chemical analysis alone to be of high organic purity. It has been shown that *Bacillus typhosus* actually

flourishes better in a water which is pure and free from other matter which has undergone nitrification (p. 105). In an inoculated water Pearmain and Moor found no less than 900,000 bacteria per cubic centimetre, but the amount of pollution produced by adding the broth culture to the water was so small as not to appreciably raise the amount of albuminoid ammonia.

Nitrites are usually looked for qualitatively by colour reactions, and are returned as strong or slight, according to the intensity of the colour produced. They are generally regarded as a bad sign when present to any appreciable extent, as they either indicate that the organic matter is only then undergoing oxidation, and is therefore recent in character, or point to a reduction of nitrates present in the water by reducing organisms and fresh contamination with organic matter. In this way a river water containing a large quantity of nitrates may suddenly lose them owing to admixture with fresh sewage, but the change is usually detected by the simultaneous production of nitrites. The presence of nitrites, therefore, indicates temporary or unstable conditions of the nitrogen contents of the water, and points either to incomplete nitrification of the ammonia, or to a reduction of the nitrates previously present.

Nitrates are present in rainwater to a very slight extent, and are derived from the air, being produced probably by the direct combination of atmospheric

oxygen and nitrogen during thunderstorms. Mainly, however, they are the product of nitrifying organisms. Dr. E. Frankland's original description of nitrates as "previous sewage contamination" is thus to a great extent justified, as moorland waters and those containing vegetable *debris* are almost free from nitrates. In deep well-waters from the chalk the nitrates are often high; here the water, originally derived from the surface, has passed through a perfect natural nitrification and filtration. But nitrification may take place in a polluted water so rapidly that nitrates may accumulate after transit through a layer of soil quite inadequate to remove the germs of either typhoid or cholera. Therefore a water which contains over 0·5 or 0·6 parts of nitrogen, as nitrates or nitrites, in 100,000 may be certified as dangerous, even if for the time the free and albuminoid ammonia are not excessive, especially if the chlorides are also present in undue proportion. The results of nitrate and nitrite determinations are usually recorded as "oxidised nitrogen."

The results obtained as above, with a microscopical examination, constitute in most cases sufficient data for an opinion on the quality of a drinking water. But as germs of disease are so excessively minute that they may be actually present, and yet give no weighable or measurable quantities to chemical analysis, the latter alone can never certify that a water is perfectly safe. A chemical analysis, however,

gives valuable information, and for the following reasons should never be omitted :—

1. Changes in the chemical composition of a water reveal the presence of active bacteria.

2. When pathogenic organisms are present in small numbers, their detection by bacteriological methods is exceedingly doubtful.

3. Bacteria do not thrive without nitrogenous food, which is at once detected by analysis.

4. Their entrance into a water supply is almost always accompanied by sewage products, which reveal themselves to the chemical examination, and in cases of doubt the chemical analysis should always be supplemented by a bacteriological test.

For domestic and industrial purposes the *hardness* of water is an important item. It also gives an insight into the mineral composition of the "total solids," whether the water contains much lime or magnesia, and whether they are present as carbonates (temporary), or as sulphates, chlorides, or nitrates (permanent hardness). The chlorine and nitrates ("oxidised nitrogen") will have been already determined; the sulphates can be tested for by comparison with a water of known composition, *e.g.*, the tap water of the place. If the total hardness be deducted from the total solids, we have approximately the amount of sodium and potassium salts, which in some samples are a leading feature, and when excessive render the water laxative, of a bad taste,

and unfit for drinking (see Table A in Appendix). The presence of *potassium* is significant in suspicious cases. Urine contains sodium salts, fæces yield mainly potassium compounds; hence the latter in large quantity point to pollution by solid excreta. *Phosphoric acid*, as a rule, is practically absent from pure waters, though traces occur where the strata contain coprolites. As phosphates are a characteristic ingredient of both urine and fæces, "heavy traces" condemn a water; "traces" are suspicious. In sewage effluents which have been treated with alum and lime, phosphates are usually absent, having been precipitated as the insoluble phosphate of alumina. They may sometimes also be low in sewage effluents and undoubtedly polluted waters, if aquatic plants have had time to remove them in their growth.

Organic carbon and nitrogen, or combustion process (Frankland and Armstrong).—The water is evaporated with certain precautions to remove the nitrates, and the residue burnt with oxide of copper. The product consists of carbonic acid and nitrogen, which are measured, and the former calculated into "organic carbon," the latter into "organic nitrogen." The relation between them reveals whether the contamination is of animal or vegetable nature, since animal matter has, as a rule, a greater percentage of nitrogen. Unfortunately, the process is liable to numerous errors, the chief of which are :—

1. During the prolonged evaporation (twelve to

twenty-four hours), destruction of the organic matter and loss of volatile compounds occur.

2. Ammonia or dust may be absorbed from the atmosphere.

3. The nitrates, especially if high, are not always completely destroyed. Any remainder would figure as "organic nitrogen."

4. Uncertainty as to how much ammonia is retained by the acid.

5. Introduction of nitrogen from the copper oxide during the combustion, of occluded hydrogen from the metallic copper, and thence the formation of carbon monoxide, either of which, if not tested for, would be returned as nitrogen.

6. Leakage of air into the pumps, &c.

7. The fallacy of deducting the amount of gas (CO_2 and N) obtained in a "blank" experiment, as a correction for air-leakage, impurity of reagents, &c., since this is an exceedingly variable quantity. Many analysts who have obtained the apparatus have consequently discontinued to use it.

Kjeldahl process, as modified by Drown and Martin. —The water is boiled down with concentrated pure sulphuric acid to near dryness, a little permanganate added, and gentle heat continued until the brown colour has almost disappeared. By this means the nitrates and nitrites are first expelled, and the remaining nitrogen is converted into ammonia, which remains as ammonium sulphate. The residual liquid

is distilled with pure soda, and the ammonia determined by the Nessler test or otherwise. After deducting the free ammonia, the rest is calculated into "organic nitrogen" (Kjeldahl). The process is a useful one: the results are about double those of the albuminoid ammonia (see p. 242).

No method at present devised yields with certainty the *whole* of the organic carbon and nitrogen in a water, and any that did so would still furnish little certain information as to its composition. Isolation of definite compounds from larger quantities of water is the direction that future analysis must take, and a few attempts have already been made.

H. Fleck (*Zeitschrift für Angew. Chem.*, 1889, 580) evaporates one or two litres to dryness with tartaric acid, extracts with absolute alcohol, evaporates, and moistens with potash solution. With polluted waters he obtained a distinct odour of fæces (*skatol*?).

M. Baudrimont extracts the original water with ether: on spontaneous evaporation of the solvent characteristic odours, fatty residues, &c., are left.

Zune concentrates the suspected water at a gentle heat until a few cubic centimetres are left, then extracts with warm alcohol. In the case of pollution by urine or fæces, he finds urea and biliary matters in the alcoholic solution, and uric acid (by the murexide test) in the insoluble portion. Such a discovery would, of course, be proof positive of admixture with fresh sewage. But these methods only apply to

recent and extreme contamination. Odours are liable to great divergence of opinion.

Products of manufacture occasionally find their way into drinking water. Soap, petroleum, various fibres, traces of metals and chemicals have been detected in domestic supplies. These occurrences have sometimes been of service, as pointing to a leakage into wells or pipes that might also admit pathogenic organisms (see p. 24). Poisonous metals, like lead, copper, and zinc, should be entirely absent. Not more than a trace of iron is admissible. Arsenic, barium, manganese, &c., have been occasionally recorded.

In an interesting recent paper (Royal Dublin Society Transactions, September, 1895), W. E. Adeney has proved that it is important in the examination of a water to show (1) the absence of easily fermentable matters of all kinds; (2) that it has been subjected to efficient natural or artificial filtration. The first condition will have been established if the water contains no free ammonia, or only slight traces, since of the easily fermentable substances present in waters it is the last to be fermented. The second is satisfied if traces only of fermented organic matter are found. To determine the rate of progress of the natural purification of polluted waters by bacteria and oxidation, he estimates the oxygen, carbonic acid, ammonia, nitrite and nitrate, present in the water, kept out of contact with air, at various stages.

The *determination of dissolved oxygen* is seldom made.

It is valuable in showing the purification or pollution of rivers during flow and in filtration experiments. Gerardin has shown that diminution in the amount of oxygen dissolved in a water indicates low *vegetable* life, and usually results in an unpleasant odour and taste, besides retarding natural purification. A fully aerated water contains about 6 c.c. of dissolved oxygen per litre, a sewage or badly polluted water none.

The following are fairly valid inferences:

Free ammonia.	Albuminoid do.	Chlorine.	Indications.
High.	Moderate.	Small.	Sewer gas.
High.	Very high.	High.	Sewer water.
High.	Rather low.	Very high.	Urine.
Rather high.	Low.	Very low.	Vegetable matter, perhaps marshy.

Dr. Smart has pointed out that, in the albuminoid ammonia process, fermenting vegetable matter gives a yellow colour with the carbonate of soda, and a greenish with the Nessler test. This, coupled with the oxygen consumed and the rate of evolution of the albuminoid ammonia, led to the following discrimination:—

NH_3 evolved slowly = recent organic matter.
  Oxygen consumed low = animal.
  ,, ,, high = vegetable.

NH_3 evolved rapidly = decomposing organic matter.
  Oxygen low: Nessler colour, the normal brown } = animal.
  Oxygen high: Nessler greenish, Na_2CO_3 yellow } = vegetable.

The above differences of colour have been for a long time observed, and have been attributed to different causes. Water containing notable amounts of sewage always gives a peculiar aromatic odour in the first albuminoid distillate.

Wanklyn's standards for albuminoid ammonia are:—

High purity, 0 to ·0041 parts per 100,000.
Satisfactory, ·0041 to ·0082.
Impure, over ·0082.

In the absence of free ammonia, he does not condemn a water unless the albuminoid exceeds ·0082, but a water yielding ·0123 he condemns under any circumstances. This would frequently, and with justice, condemn the waters of the London companies.

Frankland and Tidy's standards for oxygen consumed are:—

High organic purity, ·005.	Medium, ·05 to ·15.
Doubtful, ·15 to ·21.	Impure, over ·21.

Tables of hard and fast limits for waters are, however, useless and misleading. So much depends on the locality. A number of typical analyses will be found in the Appendix.

Bacteriological examination.—Bacteria are divided into groups, based upon their appearance under the microscope (Fig. 50), as follows:—

1. *Micrococci*, or rounded forms, seen under ordinary powers as simple dots. These may be single,

or micrococci proper; double, or *Diplococci;* in fours or cubical packets, as *Sarcina* (one form of which is common in the human stomach); in bunches, like grapes, as *Staphylococci;* or connected in chains, as *Streptococci.* Often they are collected in jelly-like "zooglœa" masses.

2. *Bacilli*, or short rods, often connected end to end to form a conferva-like line, or grouped side by side. The ends of the rods sometimes widen into dumb-bell

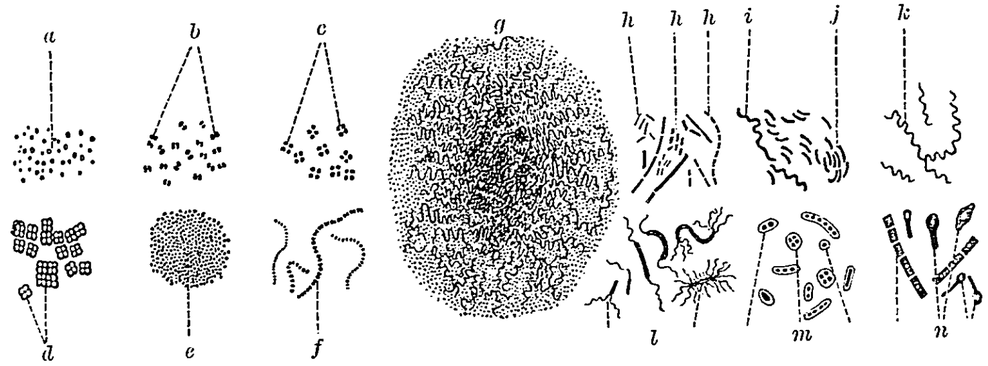

FIG. 50.—Forms of Bacteria: *a*, Micrococci; *b*, Diplococci; *c*, Tetracocci; *d*, Packet cocci (Sarcina); *e*, Staphylococci; *f*, Streptococci; *g*, Zooglœa colony; *h*, Bacilli; *i*, Spirilla; *j*, Comma bacilli; *k*, Spirochœtæ; *l*, Ciliated cells; *m*, Cocci with capsules; *n*, Bacteria showing spores.

shape, and spores may form in clear vesicles in the middle or at the ends. The rods are, in a few species, curved into "comma" or short spiral forms, which are then considered as belonging to group 4.

3. Longer unsegmented threads, straight or undulating, often matted and interlaced into flocculent

masses. *Crenothrix* (Fig. 51) develops in water-pipes and in covered tanks, under the influence of darkness and of deficient aeration, sometimes to such an extent as to communicate a bad odour and taste to the whole supply. It imparts a reddish tint to the liquid, owing to the oxide of iron which it assimilates and then excretes; it increases very rapidly by spores. At Lille and at Berlin it has caused very great trouble and expense. *Cladothrix dichotoma* also occasions great inconvenience by blocking pipes, especially if the water is periodically stagnant, as in intermittent supplies, and when it is rich in organic material. In large numbers it gives rise to whitish flocculent masses; threads of it are easily identified under the microscope, and indicate that the water is not in a proper state, or that the filtration is inefficient. It develops a strong mouldy smell, and precipitates carbonate of lime round its filaments, so that if treated under the microscope with hydrochloric acid it shows bubbles of carbonic acid gas. *Beggiatoa alba*, "the sewage fungus," occurs as whitish or grey

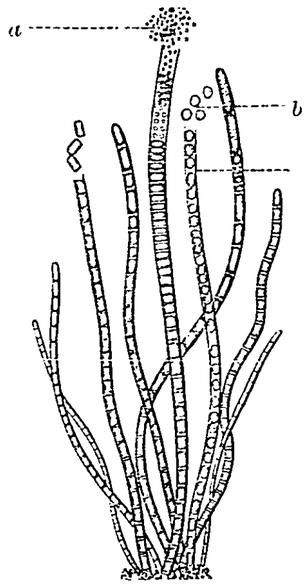

FIG. 51.—Crenothrix Kühniana (×600). *a*, Arthrospores; *b*, single segments; *c*, common sheath surrounding the separate spores.

threads, or large flakes (Fig. 52), by the sides of effluents, and sometimes finds its way into polluted drinking waters. At the extremities of the filaments highly refracting granules will be seen under the microscope; these are sulphur in a liquid state secreted by the plant, and formed either by a reduction of sulphates to sulphides or from sulphuretted hydrogen produced by putrefaction; in either case the water is obviously unpotable. This fungus is frequent in drain-water, and is also found in sulphur springs. *Leptothrix ochracea* is one of the "iron-bacteria," growing sometimes luxuriantly in ferruginous waters containing very small quantities of organic matter. It occasionally leads to rust-coloured flakes and crusts in decanters, and shows that the water contains too much iron to be wholesome, and

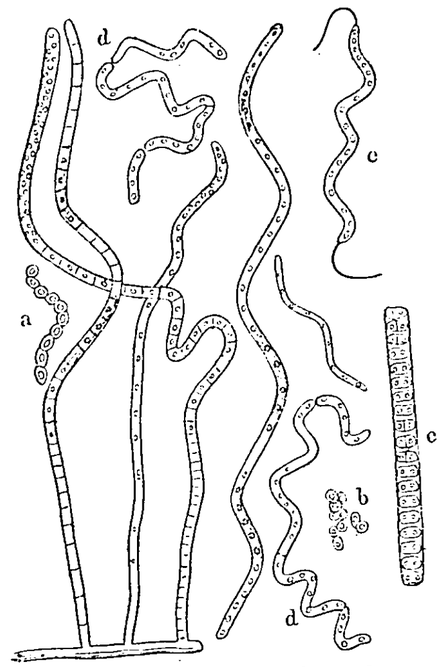

FIG. 52.—Beggiatoa alba, showing attached, free, curved, and spiral forms. *a*, chain of spores; *b*, free spores (motile); *c*, portion under a higher power, showing transverse and longitudinal division; *d*, filaments breaking up (the small dark circles are granules of sulphur highly refracting); *c*, free motile segment with terminal flagella.

indicates that the water should be previously treated by lime and deposition or filtration (p. 199). *Fusarium aquæductuum* (*Fusisporium moschatum*), the "musk fungus," was found by Lagerheim in the tap-water of Upsala as long greyish masses hanging down from the orifice of the pipes. Its presence has been suspected in many waters having a musky odour; it is believed to be pathogenic (Heller).

4. Screw-shaped or spiral bacteria. *Vibrios* are short, undulating forms; *Spirilla* are longer, and in a distinct helix or screw; *Spirochæta* is a long, thin thread, with numerous short turns of the spiral (see Fig. 50). The comma bacillus has been variously referred to Vibrio or to Spirillum, as both forms occur. This variability of shape of the same species renders it necessary to supplement a simple microscopic examination by cultivation experiments. Certain identification of a special organism depends on :—

(*a.*) The microscopic appearance at different stages of growth.

(*b.*) The presence of capsules, spores, flagella, &c.

(*c.*) Motility. Some bacteria are sluggish or almost immotile; others exhibit rapid changes of position. In this case minute, whip-like processes, called *cilia* if short and numerous, or *flagella* if few and lengthened, should be looked for by careful staining with iodine, fuschine, or other reagent.

(*d.*) The production of substances recognisable by chemical tests, such as indol (p. 268), &c., and of

characteristic colours by chromogenic bacteria, of fluorescence, of odours, of phosphorescence, of liquefaction, turbidity, precipitates, or gases.

(*e.*) Whether the bacterium can live without oxygen. The larger number are incapable of existing in absence of air, and are called *aerobic*. Such as cannot grow in the presence of oxygen are termed *anaerobic*. Both of these are described as *obligate* aerobes or anaerobes. If an organism can thrive under either condition it is said to be *facultative*.

(*f.*) The results of cultures in different media and at various temperatures.

(*g.*) Experiments by inoculation on animals. These can only be carried out under a licence, and are not necessarily conclusive as to man.

Cultivations are made in various nutrient media, some of them liquid, such as meat-broth, urine, milk, hay-infusion, white-of-egg solution, blood serum, &c., and are preserved in test-tubes closed by plugs of cotton-wool—such a plug, while admitting the air, excludes the micro-organisms floating in it — the liquids, tubes, and wool having been carefully and separately sterilised beforehand by heat. A small quantity, say one cubic centimetre, of the substance to be examined is transferred to one of the tubes, and "incubated" at a certain temperature, usually "blood-heat," about 37° to 38° C. After a time, growth will be indicated by turbidity. A minute drop of the

liquid is then transferred by a platinum wire, which has been previously heated in a flame, to a second tube of the same or another medium, and again incubated. By repetition of this treatment a pure cultivation of a particular organism can sometimes be obtained if the solutions have been sufficiently dilute.

But, by the use of solid or semi-solid media, far more distinct results are realised. The most usual are gelatine for ordinary, and agar-agar (a seaweed jelly from Japan) for higher temperatures. These are mixed with various nutritive additions, such as meat extract, peptones, glucose, salt, &c., and rendered faintly alkaline. They are filtered clear, sterilised, and, while hot, poured into test-tubes and closed, as above, and are readily portable when the medium sets. For waters the "plate method" is most useful. A shallow glass dish, with a close-fitting glass lid ("Petri's dish"), is sterilised, and the two, kept together by an indiarubber band, are taken, with a tube of the prepared gelatine, to the spot where the water is to be collected. Here a sample is taken, with the precautions described at p. 18. The gelatine is first liquefied by very gentle heating with a spirit lamp, a one cubic centimetre pipette sterilised by passing it backwards and forwards through the flame, is filled with one cubic centimetre of the water, diluted with sterile water if necessary, and then transferred to the tube, mixed with the gelatine by shaking, and poured on the plate, which is covered,

and secured by the band. The cultivation, when set (which sometimes in hot weather requires placing on a piece of ice), is fixed horizontally in the sample box and conveyed to the laboratory. In many cases it is necessary that samples should be packed in ice during conveyance; special apparatus have been devised for the purpose (see Fig. 49).

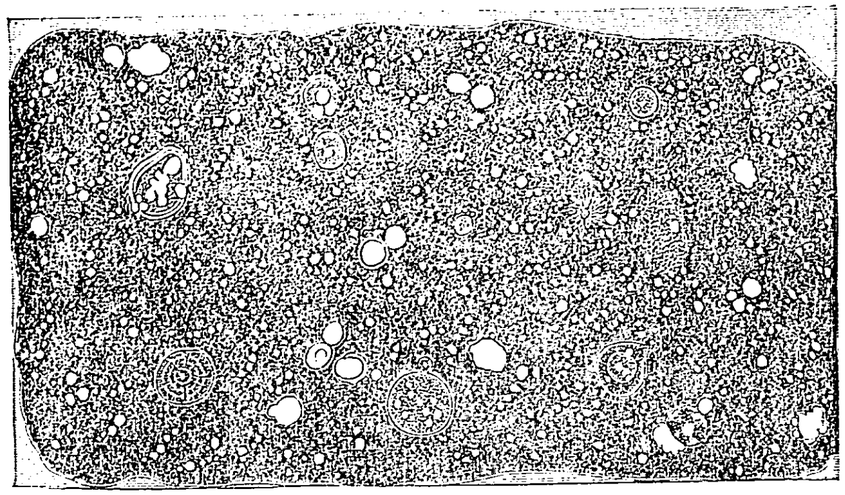

FIG. 53.—A Koch-plate culture, showing colonies.

At the laboratory the inoculated plates are kept under a bell jar in a cool room, and their changes watched. If germs are entirely absent, the gelatine will remain clear, and no spots appear on the surface but this very rarely occurs in nature. Deep well-waters, after a time, show a few isolated specks; other waters, according to their quality, show greater or less numbers of centres of growth (Fig. 53). These

are due to the multiplication of scattered organisms or their spores, and soon exhibit differences which are characteristic of individual species, and which may often be discriminated by the naked eye. Some form cup-shaped depressions of liquid gelatine, others refuse to liquefy the medium. The "colonies," as these agglomerations of growing organisms are called, are either raised above the surface or penetrate deeply into it; the outline may be ragged or circular; branchings from the centre or concentric circles may appear; they may remain white or develop peculiar pigments. Bad waters cause the gelatine to rapidly liquefy and to emit an unpleasant putrefactive odour.

Attempts have been made to render the bacteriological tests quantitative by counting under the microscope, on a glass plate ruled in squares, the number of colonies in several separate squares, then calculating the average from the number of squares on the whole plate. As each colony originates from one individual, a factor is obtained which represents the number of organisms per cubic centimetre. But it is obvious that the *number* of bacteria alone furnishes very imperfect information unless their *nature* is also known. It is, however, of great value in controlling the efficiency of filtration or the carefulness of storage, as where innocuous organisms can penetrate, disease germs can also find a way. For this reason, Koch prescribes for a good drinking water a maximum limit of 100 microbes per cubic centimetre. The

following is Miquel's experience of the numbers found in different classes of water:—

	Number of organisms per cubic centimetre.
Exceedingly pure water	0 to 10
Very pure ditto	10 to 100
Pure water	100 to 1,000
Mediocre water	1,000 to 10,000
Impure ditto	10,000 to 100,000
Very polluted ditto	100,000 to many millions.

There is no doubt that these limits are too wide, and Koch's figure, 100 per cubic centimetre, is now generally looked upon as easily reached by good filtration. Deep well-waters, as a rule, contain less than ten, while P. Frankland found that out of sixty-one samples of filtered water collected at the London companies' works only one contained more than 100 colonies per cubic centimetre, the average being twenty-nine. But the water as delivered to the consumer frequently contains a much larger number, as is shown by the table of analyses in the Appendix.

To ascertain the nature of the organisms, as soon as the growths exhibit marked characteristics, a portion of any suspected one is transferred by a sterilised platinum wire to fresh culture media, and the development watched, as already described. Among the methods are:—

1. *Streak cultivation.*—A test-tube of melted gelatine or agar is laid in a slanting position to expose a long surface; then, on solidifying, it is restored to the vertical, and the surface scratched lightly with

a platinum wire carrying a minute portion of the suspected colony (Fig. 54). Many of the pigment-producing and other bacteria develop best in the dark.

2. *Stab cultures.*—The tube is held horizontally, the inoculated wire plunged steadily nearly to the bottom (Fig. 55), withdrawn, and the wool plug at once replaced. Certain ramifying growths show themselves better under this method. Moreover, the occurrence of a growth in the deeper layers will often reveal the presence of anaerobic organisms, which can afterwards be specially cultivated, as described below.

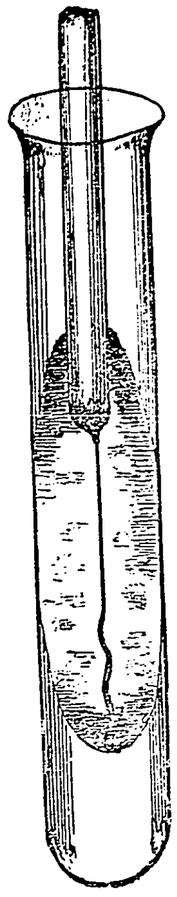

FIG. 54.
Streak cultivation.

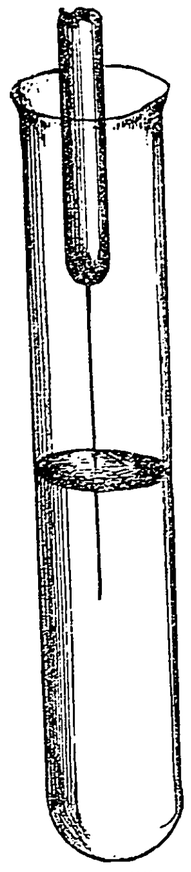

FIG. 55.
Stab culture.

3. *Roll cultures* (Von Esmarch).—A wide test-tube is partly filled with liquid gelatine, previously inoculated in the usual way, closed with a cotton-wool plug, which has been first singed in the flame, and an indiarubber cap drawn over the end. The tube is then held

horizontally in iced water, and rotated with the fingers till an even layer of the gelatine has set

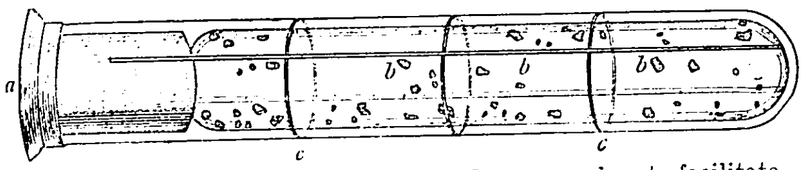

FIG. 56.—Roll culture, showing lines drawn on glass to facilitate counting.

round the walls of the tube (Fig. 56). They must be kept in a cool place.

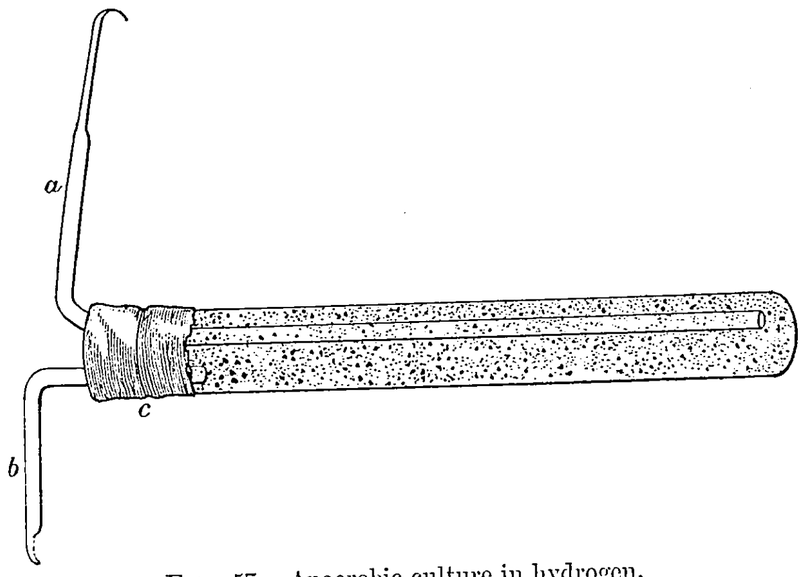

FIG. 57.—Anaerobic culture in hydrogen.

4. *Anaerobic cultivations.*—A wide test-tube fitted with two narrow tubes, as shown in the drawing, is sterilised, and the inoculated gelatine is introduced.

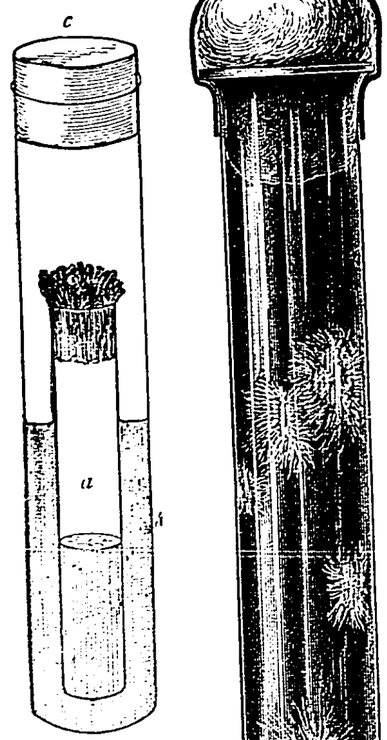

FIG. 58.
Anaerobic culture in jar.

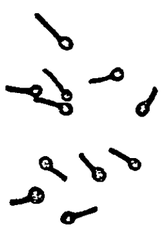

FIG. 59.
Tetanus bacilli with terminal spores.

FIG. 60.
Tube culture of Tetanus bacilli (anaerobic).

Hydrogen gas is now passed through the liquid, kept warm, until the air has been completely displaced; then the two glass tubes are rapidly sealed at the blowpipe, and the caoutchouc stopper covered with melted paraffin wax. The tube is now rotated horizontally in water for a roll culture, as above (Fig. 57). Or the culture is mounted in a closed jar containing a layer of pyrogallic acid and potash to absorb the oxygen (Fig. 58). The air may be also exhausted by an aspirator and the apparatus sealed. In this way Roux isolated the Tetanus bacillus (Figs. 59 and 60) from the filtering galleries at Lyons (p. 170), and Miquel from the waters of the Seine and Marne.

It is evident that obligate anaerobes can only live in water from which the free oxygen has been exhausted. Facultative anaerobes, moreover, flourish better where air is excluded.

Gelatine, tinted lilac by neutral litmus, is useful for detecting the formation of acid or alkaline products. The typhoid bacillus is one of those which form acids (Petruschky).

Egg albumen is a good medium for distinguishing between the different bacteria resembling that of Asiatic cholera (p. 267). Herring gelatine and boiled fish are particularly suited for phosphorescent species. Boiled rice, bread-pap, wafers, and other starchy substances, are favourable to the growth of chromogenic bacteria; while sterilised slices of potato are of great value for growing several pathogenic forms, such as that of typhoid and others.

Many of the bacterial pigments have distinct chemical reactions. Some species, like *B. coli communis*, coagulate milk; the majority do not. *B. lactis viscosus* was first found by Adametz in the water of brooks in the neighbourhood of Vienna. It is a widespread infector of milk, rendering it slimy and foul. Butter made with such milk quickly spoils. *B. butyricus* and *B. lacticus* can be carried by water, as well as many others which set up peculiar fermentations.

Staining Micro-organisms.—A drop of the culture is spread over a cover-glass with a platinum wire, and

dried by a very gentle heat. It is then held by forceps, with the residue upwards, and passed twice or thrice rapidly through a flame. Next it is floated face downwards on the staining solution, which may be methylene blue, fuschine, gentian violet, or other dye. After about five minutes the specimen is washed with water, dried by very gentle pressure between filter-paper, and examined under the microscope with a one-twelfth-inch immersion lens. Further details must be sought in special works on Bacteriology. *Moulds* sometimes develop on the gelatine plates: they do not usually occur in any quantity in waters, unless these have been improperly stored. Their appearance is generally a sign that the sterilisation has not been completely effected.

The *size* of organisms is recorded in micro-millimetres $= \frac{1}{1000}$ of a millimetre, commonly abbreviated μ. In the absence of a scale, a comparison may be made with bodies of known size, such as red-blood corpuscles.

In the "hanging drop" examination, a portion of the fluid is transferred by a loop of wire to the surface of a thin cover-glass held by forceps. This is then inverted over the well of a hollow slide, round which a ring of vaseline has been painted, so as to fix down the cover-slip. The edge of the drop must be first focussed with a low power, and then with a higher ($\frac{1}{8}$). In this way the growth of bacteria can be better observed, and their motility noticed. The cover can

at any time be removed, dried, stained, and examined under the high power.

Distilled water free from microbes is frequently required. It is obtained by a Chamberland-Pasteur or Berkefeld filter (p. 178). All vessels must be free from grease or dust, and must be sterilised before use by soaking in a one per 1,000 solution of mercuric chloride, then washing with the pure water and heating to 100° C.

FIG. 61.—Cholera bacillus.

Koch's "Comma-Bacillus," *Vibrio* or *Spirillum Choleræ Asiaticæ*, first found by him in the water of a tank at Calcutta (Fig. 61), readily multiplies in sterilised and pure waters, but in river water is soon crowded out and starved by the ordinary water bacteria, hence it has been discovered on comparatively few occasions. It appears as curved or undulating rods, mostly short, but sometimes lengthened into threads of spiral form, very motile, and ciliated at one end. On gelatine the colonies are circular, with a rough, irregular, scintillating surface and indented margins. The medium liquefies very slowly, cavities being formed by the evaporation. A stab-cultivation gradually grows as a loose, white thread, without branchings. Ultimately the whole becomes

fluid. Since it is easily killed by the presence of free acid, all media must be made slightly alkaline by carbonate of soda. On potatoes thus prepared it forms at 30° to 40° C. a greyish-brown layer. In peptone solution at 38° C. the microbe forms a pellicle, a portion of which should be examined under the microscope; it is best stained by fuschine. The peptone solution is examined at intervals for the "cholera-red reaction," by adding a few drops of hydrochloric or sulphuric acid, when the rose colour of nitroso-indol appears. This reaction depends upon the fact that the microbe produces indol, *as well as nitrites*, whereas nearly all those that resemble it do not show the same chemical action. *B. coli communis* also forms indol, but not nitrites, consequently it does not give the colour unless nitrite is also added, whilst *B. typhosus* does not form indol.

Gruber uses a comparative method. He prepares a number of tubes containing cholera microbes in peptone grown at 38° C., then sterilises them by heating for ten minutes to 65° C. A number of such tubes are inoculated with the suspected water and kept at 38° for twenty-four hours. One is tested for cholera-red; if it gives a deeper colour than a tube that has not been inoculated, it proves that the water contains an organism similar to that of cholera, which has continued the indol-formation which was interrupted by the death by sterilisation of the previous cholera

vibrios. Other inoculated tubes are examined under the microscope and by cultures. Klein says that it is possible to give a definite opinion in from eighteen to forty-eight hours. The best proportions are 1 or 2 per cent. peptone, 0·5 per cent. sodium chloride, and quantities of the suspected water diminishing in different tubes from an equal amount down to a quarter of a cubic centimetre in the sub-cultures on agar-agar plates. These latter are better placed in the incubator with the lid downwards, so that the condensed moisture does not fall on the surface of the medium. The gelatine sub-cultures are maintained at 22° C. The colonies show their characters in thirty-six to forty-eight hours. Koch also relies on the pathogenic effects on guinea-pigs (*cobayes*), which are affected by the cholera vibrios, but apparently not by the allied forms. It is believed, however, that there are a number of different organisms which at stages of their development can produce in man the symptoms of cholera, some of them giving the "cholera-red" reaction. In a search for cholera organisms in water, the sample must be examined at the earliest possible moment after it is taken, and light should be excluded.

The *B. typhosus* of Eberth appears as short, plump rods with rounded ends, growing sometimes in cultures into long threads (Fig. 61A). They are extremely motile, surrounded on all sides by a great number of cilia, so as to present, when stained, the

FIG. 61A.—Typhoid bacilli.

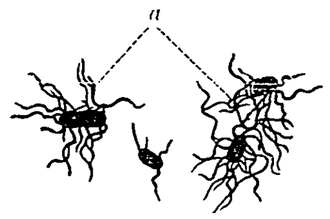

FIG. 62.—Typhoid bacilli (spider forms showing *a* flagella).

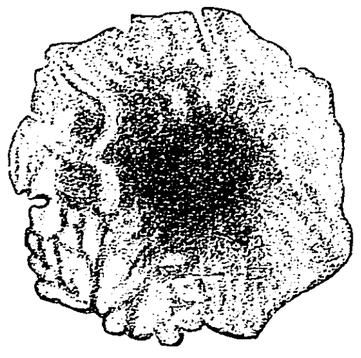

FIG. 63.—Colony of B. typhosus on gelatine plate, five days old.

appearance shown in the figure (Fig. 62); plate colonies are whitish, with indented margin, becoming yellowish-brown and not liquefying.

B. coli communis is constant in the intestines of man and animals, and will always be present where typhoid is suspected. It has often been found in waters, and is a certain sign of pollution by excreta. It forms short rods, sometimes in pairs, feebly motile, and with one to three flagella. Gelatine colonies resemble those of typhoid (Fig. 63). On potatoes it generally forms thicker and yellowish expansions. A thrust culture in gelatine always develops large bubbles of gas, while when grown in peptone broth it yields with sulphuric acid, *after the addition of a little*

sodium nitrite, a red indol colour. *B. typhosus* gives neither of these reactions.

B. coli communis introduced into milk which has been sterilised coagulates it after twenty-four to forty-eight hours at 38° C.; *typhosus* does not. Both *coli* and *typhosus* are capable of growing in a "carbolised gelatine" containing 0·05 per cent. phenol, while almost all other organisms, particularly the liquefying ones, are not. Therefore the following method for isolating the two species from others is adopted.

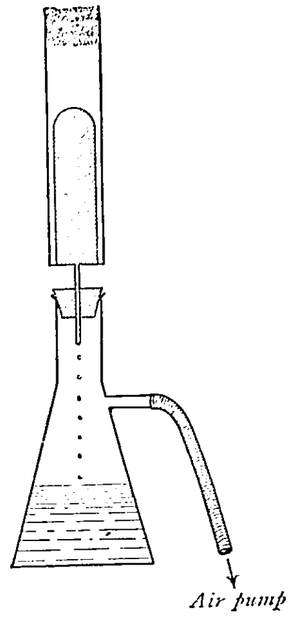

FIG. 64.—Diagram of bacterial filtration.

A litre of the water is drawn by an air-pump through a sterile Pasteur or Berkefeld filter into a sterilised flask (Fig. 64). The "candle" is now unscrewed, and the film of bacteria, &c., brushed off into about ten cubic centimetres of the filtrate in a small beaker. From 0·1 to 0·25 cubic centimetres of this liquid is transferred to test-tubes containing sterile gelatine, with 0·05 per cent. of phenol and 0·05 per cent. of hydrochloric acid, thoroughly mixed, poured into Petri's dishes, as already described, and allowed to remain for twenty-four to forty-eight hours. Any colonies presenting the appearance of *coli* or *typhosus*

are used for (*a*) gelatine streak cultures; (*b*) gelatine shake cultures; (*c*) 25 per cent. gelatine stab cultures. Place (*a*) and (*b*) in the cool incubator, and (*c*) in the warm, at 38° C. Tube or plate cultures which show signs of *typhosus* or *coli* are subjected to microscopical examination, the milk-test, peptone cultivation and the indol-test, growth on potatoes, &c., looking also for the formation of gas bubbles. By taking separate colonies and growing them,

FIG. 65.
Spirillum undula.

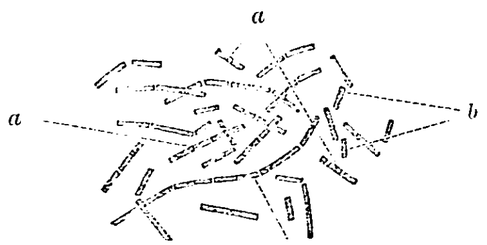

FIG. 66.—Bacillus anthracis. *a*, Spores; *b*, detached short rods.

pure cultures of each organism can ultimately be obtained.

B. coli itself is believed to be pathogenic in certain stages of development. In any case its presence is condemnatory of a water, since it points to past or present fæcal contamination. Dr. Klein, for example, has found this organism present in one week in four out of the eight London companies, and in two other weeks it was found in two. The Southwark, the East London, and the Lambeth all yielded it, in two weeks out of eight, in the early part of 1895.

Spirilla are frequently present in stagnant waters, and are characterised by their rapid motion, due to the flagella with which they are provided. Some of these may be pathogenic; *Spirillum undula*, the common form, according to Schenk, is shown in Fig. 65.

B. anthracis, which has been identified in one or two cases in water, has the appearance shown in Fig. 66.

Many other disease germs only flourish at the temperature of the body, and would therefore not exist for long in the water.