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MICRO-ORGANISMS IN WATER

MICRO-ORGANISMS IN WATER

THEIR SIGNIFICANCE, IDENTIFICATION AND REMOVAL

TOGETHER WITH AN ACCOUNT OF THE BACTERIOLOGICAL METHODS EMPLOYED IN THEIR INVESTIGATION

SPECIALLY DESIGNED FOR THE USE OF THOSE CONNECTED WITH THE SANITARY ASPECTS OF WATER-SUPPLY

BY

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PREFACE

The existing literature in connection with the Bacteriology of Water is so extensive, and is scattered through such numerous publications both English and foreign, that we venture to think an urgent necessity has arisen for a work in which this subject is specially discussed. We have, therefore, in the following pages endeavoured to present in a connected form an account of the more important investigations which have been carried out in this department of Bacteriology, in the hope that it may prove of service both to the student and investigator, as well as to those who, like engineers and medical officers of health, are practically concerned with the hygienic aspects of water-supply.

With this object in view, we have given—firstly, a survey of all the more important general methods of bacteriological study, describing in detail those which are specially applicable to the examination of water; secondly, we have given an account of the principal results hitherto arrived at by the use of these new bacteriological methods in the study of the different kinds of water, and the changes which they undergo through natural and artificial agencies; further, particular attention has been bestowed on the behaviour

of pathogenic bacteria in water, whilst a separate chapter has been devoted to the comparatively novel subject of the bactericidal action of light. Finally, we have appended a concise description of the principal characters of all the micro-organisms, numbering upwards of 200, which, as far as we have been able to ascertain, have hitherto been found in water; and it is hoped that this descriptive catalogue of water bacteria may prove of some value in the at present bewildering task of identifying the different microbial forms met with in natural waters.

P. F. F.
G. C. F.

DUNDEE: *April*, 1894.

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INTRODUCTION

Until about twenty-five years ago it was generally supposed that some organic substances in the act of undergoing decomposition are capable of causing the alteration and decay of other organic substances with which they are placed in contact, and it was by the assumption of such communicated decomposition from one substance to another that Liebig sought to explain the various well-known phenomena of fermentation. Thus Liebig conceived of the ordinary alcoholic fermentation of sugar as being brought about, not by the living and growing yeast-cells, but, on the contrary, by the dead yeast undergoing decomposition. As long as this theory was the accepted doctrine of the day, it was not surprising to find chemists attaching great importance to the organic matter in water which analyses revealed, and which was known to have been derived from decomposing vegetable and animal substances with which the water had been in contact. It was not unnaturally supposed that such decomposing organic matters, if present in drinking water, would tend to set up putrefactive and other injurious changes in the digestive organs with which they were brought in contact. But the theory, or rather dogma, of fermentation

enunciated by Liebig was completely broken down by the classical researches of Pasteur, by whom it was shown that the processes of fermentation and putrefaction were due, not to decomposing organic matter, but to living organisms, and that living organisms were also certainly the cause of some, and probably of all, zymotic diseases. The presence of organic matter in water was thus deprived of much of its direct import, the chief interest still attaching to it being that it might serve as an *indication* of the possible presence of living organisms endowed with virulent properties, whilst the interest attaching to the presence of micro-organisms in water was further greatly enhanced by the proof which was furnished by medical men that some zymotic diseases are undoubtedly communicated by drinking water. In the case of two diseases, at any rate, the evidence may be regarded as conclusive on the main point, and the communicability of Asiatic cholera and typhoid fever by water forms one of the cardinal principles of modern sanitary science, which year by year is becoming more widely recognised and more generally accepted. The germ theory of zymotic disease, which has gained with each successive decade of the past half-century a firmer hold on enlightened public opinion, was naturally soon impressed into the service of those who sought to explain the empirical fact that these particular diseases are frequently communicated by water.

Having thus become so early interwoven with the consideration of potable waters, it is easy to understand how the acceptance of this germ theory of disease

caused the rapid development of our knowledge of micro-organisms in general to be followed with such eager interest by all who had to devote much attention to the sanitary aspects of water-supply.

The publication by Koch, a little more than ten years ago, of his beautiful and comparatively simple methods of bacteriological study gave an impulse to investigation in this direction throughout the civilised world, and the possibility was at once opened up of approaching the solution of problems connected with water-supply which had long been matters of dispute and speculation amongst hygienic authorities.

MICRO-ORGANISMS IN WATER

CHAPTER I

STERILISATION AND THE PREPARATION OF CULTURE MEDIA

As the whole success of bacteriological experiments depends primarily upon the absolute sterility of both apparatus and materials employed in their cultivation, it is appropriate in the first instance to briefly describe the methods of effectually removing all organisms from the objects—solid, liquid, and gaseous—which are used in bacteriological investigations. This sterilisation, as it is called, may be accomplished in various ways: (1) *by moist heat*; (2) *by dry heat*; (3) *by chemical means*; whilst liquid and gaseous substances may in addition be sterilised by (4) *filtration*.

(1) *Steam or moist-heat sterilisation.*—This is most conveniently effected by means of the 'steam steriliser,' devised originally by Koch, Gaffky, and Löffler, and which has the exceedingly simple form represented in the figure below. The cylinder is covered with felt or asbestos, to prevent loss of heat, and the bottom is constructed of copper, under which are placed one or more bunsen or other smokeless burners, according to the size. The heat applied must be sufficient to keep the water in such vigorous ebullition that steam issues

freely from the top. In this way a uniform temperature of 100° C. is obtained, and for the sterilisation of glass and other vessels an exposure of from one to two hours is necessary. In the case of some culture materials, however, such prolonged heating to so high a temperature would cause serious alteration, and the practice of *intermittent sterilisation* (Tyndall) is then resorted to. This method is also of importance in the case of those materials, such as potatoes, in which one exposure only to this temperature does not actually ensure sterility. Tyndall's plan is to submit substances to a high temperature for a short time on three to five successive days, and in the preparation of culture media containing gelatine this discontinuous sterilisation is of primary importance, as prolonged steaming greatly reduces the melting-point of the gelatine. Sterilisation may also be effected by simply boiling the articles in water; thus in the disinfection of instruments—syringes &c.—used in animal inoculations this may frequently be resorted to with advantage.

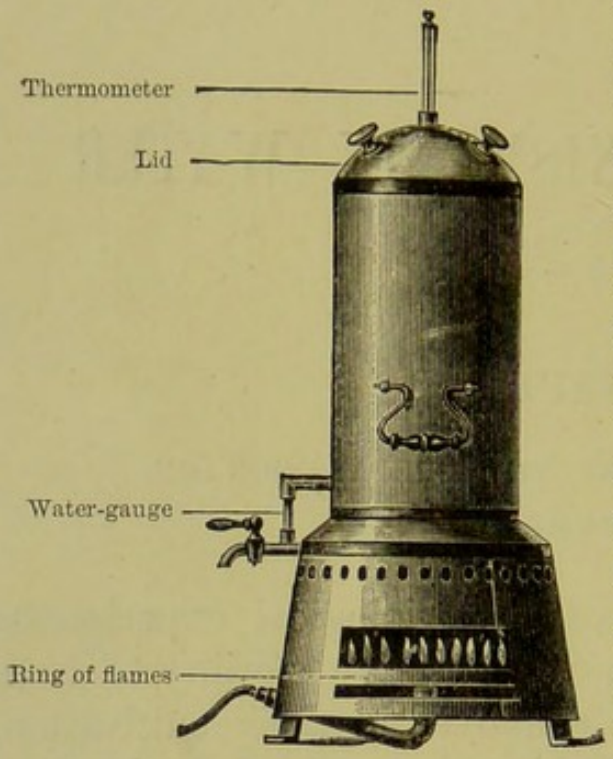


Fig. 1.—Koch's Steam Steriliser.

In some exceptional cases, *e.g.*, in the sterilisation of soil, high-pressure steam may be very conveniently used; thus certain spores were found by Globig ('Zeitsch. f. Hygiene,' iii. [1887], p. 332) to resist the ordinary process of steaming for upwards of three hours, whilst

they were destroyed in fifteen minutes by steam at 110–120° C. Various modifications of the well-known Papin's digester have been devised for use in the bacteriological laboratory, of which one of the best is that shown in the accompanying figure.

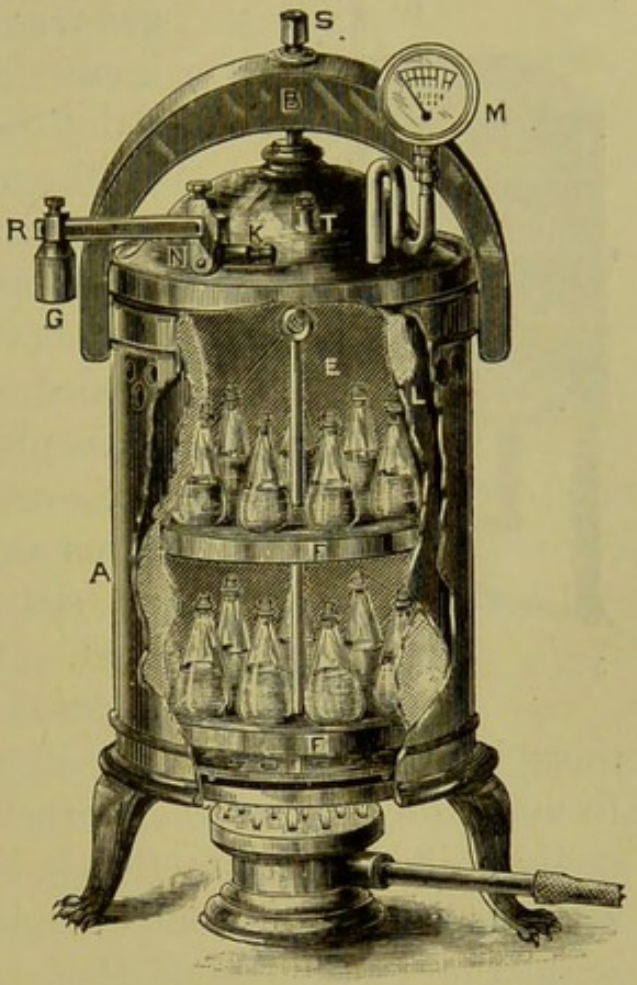


Fig. 2.—High-pressure Steam Steriliser (Muencke).

A, external jacket-wall of boiler; B, steel clamp, with screw S for brass lid K; safety valve N, with lever R, and adjustable weight G; pressure-gauge M; T, aperture into which thermometer can be screwed; L, internal wall of copper boiler; FF, brass removable stand surrounded with copper gauze E.

Owing to the much more costly and cumbrous nature of such high-pressure steamers their employment is not to be recommended for general purposes, whilst in the sterilisation of a number of substances, such as sugars, albuminoids, urea, &c., a temperature above 100° C. is inapplicable in consequence of the chemical changes brought about.

In the case of some materials, such as milk, it may be necessary to effect sterilisation below 75° C., *i.e.*, below the temperature of coagulation of albumen; and in such cases discontinuous sterilisation at 58–65° C. is employed for one to two hours on five to eight days; to this, however, subsequent reference will be made in connection with the preparation of culture media.

(2) *Hot-air sterilisation.*—The accompanying figure shows the appearance of a hot-air sterilising oven as

commonly used for bacteriological purposes. A thermometer is fitted into A, and by means of the perforated slide B, together with the gas-regulator placed in C, the temperature can be kept under strict control. This is of course of importance in the case of some substances, such as milk, where it is desired that a particular temperature should not be exceeded. Test-tubes, pipettes, glass plates, and other glass vessels, and certain pieces of apparatus as well as cotton-wool, are all sterilised by means of dry heat. Glass vessels should be exposed to 150° C. for two or more hours, and should be allowed to cool in the oven to avoid the risk of their cracking by being too suddenly chilled. In practice it is a good plan to place some loose cotton-wool in a beaker in the oven along with the glass vessels that are being sterilised, for when the former becomes slightly browned it may be taken as a sign that the sterilisation of the objects is complete, and the gas may be turned out.

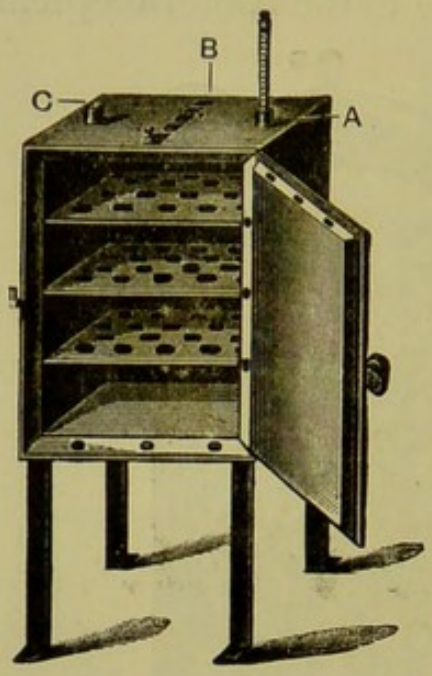


FIG. 3.—HOT-AIR STERILISER.

It is particularly important to bear in mind that the temperature is by no means uniform throughout such ovens, and care must be taken that the objects are so placed as to be really exposed to the desired temperature.

(3) *Sterilisation by means of filtration.*—In order to deprive a liquid which is not viscid of micro-organisms, it may be made to pass through cylinders constructed either of unglazed porcelain (Chamberland) or of baked infusorial earth (Berkefeld). In the case of water,

which for experimental purposes is required to be sterilised without chemical change, these Chamberland and Berkefeld filters are extremely useful (see experiments made by Percy Frankland on the vitality of the anthrax bacillus in various waters, p. 314; the sterility of the water was secured by means of a Chamberland filter). In those cases in which it is desired to separate the bacterial products from the micro-organisms themselves such filtration is invariably resorted to. Bitter[1] has made experiments on the filtration of liquids turbid from the growth of bacteria, and also of albuminous fluids, by means of the Berkefeld filter. He has found that even the bacillus of mouse septicæmia, which is one of the smallest known organisms, growing in broth, is entirely removed when the latter is passed through the above filter. Experiments were also made with blood-serum, and it was found that even putrid blood-serum may be not only quickly clarified, but the organisms entirely removed by its means. For this purpose it is best to use the more porous cylinders. Such a cylinder was found capable of filtering 680 c.c. in twenty-five minutes. In the case of fresh serum a cylinder of similar construction yielded in thirty minutes 800 c.c. of clear sterile serum. It is necessary to frequently wipe the cylinder whilst the filtration is going on. Milk may also be deprived of all its fat and a clear sterile serum obtained by filtration through such porous cylinders. For the subsequent cleansing of the filter see p. 176. This method of sterilisation, therefore, may be used with great advantage for many laboratory purposes.

Gases, again, are readily deprived of any micro-organisms they may contain in suspension by passage

[1] 'Die Filtration bacterientrüber und eiweisshaltiger Flüssigkeiten durch Kieselguhrfilter,' *Zeitschrift für Hygiene*, x. 1890, p. 155.

through a plug of sterile cotton-wool; this principle is extensively employed in the preservation of culture media in flasks, test-tubes, &c., which are protected from aërial microbes by their mouths being closed with sterilised cotton-wool stoppers.

(4) *Sterilisation by chemical agents.*—As in all operations in which chemical substances are used for sterilisation purposes the risk is incurred of traces of such disinfectants escaping removal, and so destroying the organisms under investigation, along with those foreign forms which it was desired to eliminate, extreme caution must be exercised in the employment of such substances. It is, in fact, only advisable to resort to them under exceptional circumstances, and in all ordinary operations to depend upon the conscientious fulfilment of all the minutiæ required in sterilisation by the usual methods described. In the case of experiments on animals, on the other hand, a solution of corrosive sublimate must be used for locally washing the body before making an incision, either for the purposes of inoculation or dissection in the autopsy; but in the usual routine of bacteriological investigations the use of this and other disinfectants is not only unnecessary, but attended with the very greatest risk for the reasons specified above.

CULTURE MEDIA

For the cultivation of all micro-organisms more or less moist materials are necessary, both liquids and solids being employed for the purpose. It might be supposed that it would be easy to find a medium which would suit the requirements of all micro-organisms, since from some points of view they are all so similar; but, as a matter of fact, there is the greatest diversity in their tastes, and media which are suitable for the

growth of some are utterly unfitted for the cultivation of others.

Thus, whilst some organisms are unable to thrive and multiply excepting when surrounded with the most nutritious and subtle foodstuffs, others absolutely refuse to grow unless bathed in a liquid from which such organic materials have been most carefully banished. The ingenuity of the bacteriologist is, in fact, severely tried in endeavouring to cater for the organisms which he has under his charge, and every year, or even month, sees many additions to the menu from which he has to select, and which already includes such a medley as living animals, blood serum, bouillon, beef-jelly, agar-agar, potatoes, numerous purely mineral solutions, &c. In some cases, moreover, it is necessary that these food materials should be varied from time to time, or degeneration of the vitality of the micro-organisms which are cultivated on them often takes place.

We shall now describe the preparation of some of the more important culture media in detail.

Solid culture media.—To Robert Koch belongs the honour of having adapted solid media to the cultivation of micro-organisms; for although such media had been used previously, it is the particular methods of employing them devised by Koch which have secured for these solid culture materials the extended application and universal reputation which they now enjoy; and it is by these methods that such brilliant results have been achieved in so short a period of time.

Already in the year 1881 Koch observed that if a slice of cooked potato was exposed to the air, and afterwards preserved at a suitable temperature in a damp chamber, small isolated dots began in the course of a few days to make their appearance. Of these little

centres many seemed to be different varieties, some being yellowish, brown, red, white, grey in colour, whilst in shape they also presented wide divergences.

On microscopically examining the nature of these centres he ascertained that each consisted of one kind of micro-organism; that some, for example, were made up of large micrococci, some of small micrococci, some of bacilli, and so on; that, in point of fact, *each centre was in reality a colony or pure cultivation of one particular organism.*

If, instead of a potato, a surface of liquid culture material, in area equal to that of the potato, was exposed to the air, Koch found that, although undoubtedly similar organisms gained access to the liquid as to the potato, yet their development proceeded in a very different manner. Thus, on submitting a portion to microscopic examination, the liquid was found to be teeming with all sorts and shapes of organisms mixed up one with the other in inextricable confusion, there being not the faintest approach to anything which could be designated a pure cultivation.

The difference in the development of the micro-organisms in the two instances was not far to seek, in the one case the culture material being solid, those individual bacteria which gained access to it were imprisoned by rigid surroundings, and, being unable to move from the spot, commenced to multiply, there yielding in course of time a colony visible to the naked eye. On the other hand, those which were collected in the liquid had no such restrictions imposed upon their movements, and, being free to traverse the whole extent of the liquid, multiplied indiscriminately in all directions, and hence the medley of forms which was visible under the microscope. Here, then, we have the first observations which led Koch to the elaboration of his beautiful

process of gelatine-plate cultivation, which has been the means of separation and individualisation of so many micro-organisms, and to which the science of bacteriology is so largely indebted.

It was, of course, essential that the culture medium employed should be of such composition as to afford food material for the largest number of micro-organisms, and Koch, after numerous experiments, found that a mixture of gelatine and broth gave the best results for general purposes. The original recipe recommended by Koch is, with a few modifications, that which is still in use.

In all investigations connected with micro-organisms it must be always borne in mind that all our surroundings are more or less infested with living forms of the same order as those with which we are dealing, and it becomes, therefore, of paramount importance that every operation should be conducted in such a way as to preclude the possibility, or at any rate reduce to a minimum the chance, of introducing micro-organisms from foreign sources. The precautions to be taken cannot be too scrupulous or painstaking, and if in the description of the various bacteriological processes too much emphasis may appear to have been laid upon the dangers arising from contamination through imperfectly sterilised vessels &c., the student must remember that by neglecting even the most trivial of such precautions he may very possibly afterwards discover that the whole of his labour has been rendered worthless, and that to begin *de novo* is his only alternative.

Preparation of gelatine-peptone.—The preparation of gelatine-peptone, which is in many respects the most important of all the culture media, may be best carried out in the following manner :—A pound of beef, as free

from fat as possible, is finely minced and infused with one litre of cold distilled water and allowed to stand for twenty-four hours in a cold place (in hot weather it should be placed in a refrigerator); the whole mass is then strained through linen, as much of the liquid being pressed through as possible, distilled water being added, if necessary, to restore its volume to a litre. To this clarified liquid are then added 100 grms. of French leaf gelatine, 10 grms. of dry peptone, and 5 grms. of common salt, after which the whole is placed in the steamer for about one hour until the complete solution of the gelatine and peptone has taken place. The resulting liquid, which exhibits a distinctly acid reaction, must be carefully neutralised and rendered faintly alkaline with a solution of carbonate of soda.[1] The faintly alkaline liquid is then clarified by mixing with it the whites of two or three eggs, along with the broken shells, the whole being again placed in the steamer for from fifteen to twenty minutes. The coagulated albumen rises to the surface, and carries with it the other solid particles suspended in the liquid. On then straining through linen a fairly clear liquid is obtained, which is finally clarified by passing through ribbed filter-paper, the filtration being most conveniently carried on in the steamer, which should, however, be only gently heated. The filtrate must be rejected until it runs perfectly clear and limpid. The filtrate is collected in a flask, subsequently plugged with sterile cotton-wool, and on cooling sets to a straw-coloured transparent jelly. Whilst still liquid it is poured into test-tubes which have been previously sterilised and plugged with sterile cotton-wool. The most convenient quantity to take for each tube is 10 c.c., and it is best to pour the gelatine from the flask into a small measur-

[1] In connection with the neutralisation of culture media see pp. 63-66.

ing-tube, from which it is then transferred to the test-tube. Care must be taken that the gelatine does not come in contact with the sides of the test-tube near the mouth of the latter, as this would cause the cotton-wool stopper to stick to the glass and make it troublesome to remove. To prevent this the gelatine should be poured from the measuring-tube into the test-tubes through a small sterilised glass funnel; and this, if carefully done, will prevent the collection of any trace of gelatine in the region of the stopper. After the tubes are filled, and the stoppers replaced, they are at once steamed for ten to fifteen minutes, which is repeated on the two following days.

To prepare sterile test-tubes for the reception of the jelly new tubes should be soaked in hydrochloric acid, carefully washed with a brush, rinsed out with distilled water, inverted to drain, then placed in a wire basket and thoroughly dried in the hot-air oven (see fig. 3), after which they are plugged with sterile cotton-wool and then exposed to a temperature of from 140° to 160° in the oven for from two to three hours. The cotton-wool used should be sterilised before plugging the tubes by exposure to the same temperature in the hot-air oven for two or three hours, until it becomes slightly browned, This separate sterilising of the tubes and cotton-wool may appear tedious, but it cannot be successfully dispensed with.

Gelatine tubes thus prepared may be kept untainted for an indefinite period of time.[1]

[1] It must not be supposed that the gelatine-peptone medium thus prepared will retain its properties absolutely unaltered for an indefinite length of time, although for most purposes it may be used even months after preparation. To reduce the chance of alteration in the gelatine it should always be preserved in darkness, and before use the reaction should be invariably tested, as it has a tendency to become acid on keeping. This matter is of special importance in those cases in which

If the gelatine is not required for immediate use, or if after filling tubes some remains in the flask, the cotton-wool stopper must be securely replaced and the flask sterilised again by heating in the steam steriliser on three successive days in the manner previously described. An india-rubber cap should then be drawn over the cotton-wool stopper and the flask placed in a dark cupboard and protected from dust as much as possible. Before filling the tubes the gelatine must be gently heated in the steriliser until it is liquid.

The gelatine culture medium if properly made should look perfectly clear and nearly colourless in the test-tubes, and should only melt at a temperature of 25° C.; if this occurs at a lower temperature, then it shows that the gelatine has been overheated in the preparation.

Glycerin-gelatine peptone.—It is of course possible to vary the composition of this gelatine-peptone by the addition of other substances. By adding 5–8 per cent.

any quantitative value is to be attached to the colonies of micro-organisms developing on a gelatine plate, as in the examination of waters.

Another point to which special attention must be given is that the gelatine should be preserved in test-tubes only for as short a time as possible, as owing to the large amount of evaporation going on the medium rapidly becomes concentrated and its culture properties altered. This concentration may, to a great extent, be prevented by covering the cotton-wool stoppers with an india-rubber cap, but in laboratories where much work is going on this is almost out of the question, and it is far more convenient only to fill as many test-tubes as are required for rapid use.

That the length of time a particular culture material has been prepared before use may in some cases exert an important effect upon the growth of micro-organisms has been strikingly exhibited by Kitasato,* who found that for the satisfactory development of the tetanus bacillus in broth, the latter must only be used when quite freshly prepared, for in broth, say a week old even, the bacillus will only grow slowly and badly, and the toxic products elaborated were much weaker in their action than when quite fresh broth was employed.

* 'Experimentelle Untersuchungen iibe das Tetanusgift,' *Zeitschrift für Hygiene*, vol. x. 1891, p. 280.

of glycerin before the jelly is finally sterilised a most useful culture medium is obtained. This material has the advantage of remaining moist for a much longer time than the gelatine peptone, and, whilst most organisms thrive especially well upon it, some, like the bacillus tuberculosis, refuse to grow on gelatine without its addition. Other materials, such as grape sugar, mannite, acetic acid, have also been added in different proportions for special purposes (see also pp. 63–73 in Chapter III. on the Examination of Water for Bacteria).

The following gelatine preparations may be more especially referred to :—

Gelatinised milk-serum.—One litre of fresh milk is warmed to 60° or 70° C., 70 to 100 grms. of gelatine are then added and dissolved. A few minutes' boiling will serve to precipitate the casein, and it is then passed through a fine muslin strainer. The fluid is allowed to stand for about twenty minutes at the temperature of the body, in order to allow the fat to come to the surface, after which it is allowed to cool and the layer of cream is carefully removed. To the resulting slightly opalescent fluid 1 per cent. of albumen peptone is added, and the whole is neutralised, boiled, filtered, and sterilised in the same way as nutrient gelatine.[1]

Wort gelatine and Wort agar.—These culture materials are useful for the growth of those organisms which, like yeasts and moulds, are favoured by an acid medium. To ordinary malt wort 10 per cent. of gelatine is added and the mixture heated in the steam steriliser for some time, and then filtered without being neutralised. The acidity which the medium thus acquires is rather greater than that of the wort before the addition of

[1] Hueppe, *Die Methoden d. Bakterienforschung*, 1891, p. 248.

the gelatine, but if the original acidity of the wort is required the excess must be carefully neutralised by means of sodium phosphate. For wort agar the mode of preparation is quite similar, excepting that 1 to 2 per cent. of agar is substituted for the gelatine. Under the examination of water for brewing purposes (see p. 67) an account will be found of the various culture fluids used by Hansen and his pupils for this purpose.

Miquel's high-temperature jelly.—The discovery was made by Globig of some organisms which apparently would not grow at a temperature below 50° C., and Miquel has devised a culture medium which will remain solid at this high temperature. For this purpose from 300 to 400 grams of Irish moss (Caragheen, *Fucus crispus*) are placed in 10 litres of water and heated for several hours at 100° C.; the liquid is then poured through a sieve, the filtrate boiled up again, and strained whilst hot through fine linen. The filtrate is slowly evaporated on a water-bath, and is then placed in porcelain dishes and dried at from 40° to 45° C. Miquel states that 1 per cent. of the gelatinous substance thus obtained on being added to broth yields a culture material remaining solid at 50° C.

Iron gelatine and iron agar.—In order to determine the production of sulphuretted hydrogen by certain bacteria, Fromme [1] prepared a culture material consisting of gelatine peptone, to which a small quantity of iron (the exact quantity is not specified) was added. The iron is added in the form of a 3 per cent. solution of ferrous tartrate, acetate, or saccharate to the gelatine, and the material assumes a faint reddish colour. Fromme cultivated various pathogenic organisms

[1] *Ueber die Beziehung des metallischen Eisens zu den Bakterien.* Marburg, 1891.

successfully upon this iron-gelatine, amongst which he mentions the malignant œdema and the typhoid bacillus in particular as producing such quantities of sulphuretted hydrogen in this medium that, not only did the gelatine in the immediate vicinity of the growth become black, but the whole of the culture material as well (see also p. 410 in tabulated description of typhoid bacillus).

In order to cultivate organisms in this material at a higher temperature it is necessary to use agar-agar. In addition to the iron solution a small quantity of a 5 per cent. solution of sodium sulphate and some glycerin should be added.

Fromme states that he was unable to discover any organisms in the Marburg water which produced sulphuretted hydrogen in any marked quantity, although this was not regarded by any means as an unimpeachable supply, and must have contained along with the ordinary water bacteria many organisms derived from polluting sources.

Another method of qualitatively testing for the production of sulphuretted hydrogen by bacteria in liquid media is the well-known one and that employed and recommended by Stagnitta-Balistreri.[1] Pieces of lead-paper are suspended in the inoculated flasks or test-tubes containing broth with or without peptone, and the change in the colour produced noted from deep black to pale brown. During the investigations the lead-paper must be daily observed, as, although the reaction may be distinct at one time, it may subsequently disappear.

An interesting table is given of the amount of

[1] 'Die Verbreitung der Schwefelwasserstoffbildung unter den Bakterien,' *Archiv für Hygiene*, 1892. See also 'Beiträge zur Biologie der krankheitserregenden Bakterien, insbesondere über die Bildung von Schwefelwasserstoff,' Petri and Maaszen, *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. viii. 1892, p. 318.

sulphur normally present in a litre of the various culture media employed, thus :—

Culture media	Sulphur	Amount of sulphuretted hydrogen capable of being produced
Broth	0·0705 gram	0·0749 gram
Broth with peptone . .	0·2131 „	0·2264 „
Agar-agar [1] . . .	0·3016 „	0·3205 „
Gelatine [1] (10 per cent.) .	0·7051 „	0·7492 „

[1] As used for purposes of cultivation.

The composition of the culture materials is of course by no means absolutely constant, for the extraction of the juices from the beef must vary as well as the proportion of pure muscular material present in the latter.

Agar-agar.—For the preparation of agar-agar jelly 20 grams of agar-agar is employed in the place of the 100 grams of gelatine used in the ordinary gelatine-peptone medium described above. The agar-agar must be cut up into as small pieces as possible, as this materially facilitates its solution.

The composition of the nutrient agar medium is—1 litre of beef extract, 20 grams of agar-agar, 10 grams peptone, 5 grams common salt. This mixture is heated for a number of hours in the steam steriliser until the agar-agar has completely dissolved. The liquid is then rendered faintly alkaline with carbonate of soda, clarified with white of egg, strained through a piece of linen, and finally filtered, all exactly in the same way as already described for the gelatine peptone, excepting that the filtration of the agar-agar may be carried on at a higher temperature in the steam-steriliser, as the melting-point of the agar-agar is not thereby reduced. When in a fluid condition the agar looks transparent and of a rather darker yellow-brown colour than gelatine, but when it solidifies it loses its complete trans-

parency. As agar-agar has a much higher melting-point than gelatine, it is extremely useful in the case of cultivations which require to be kept at a high temperature; but in consequence of the separation of water which takes place on its surface after it has congealed, it does not lend itself so satisfactorily to plate cultivations, as the colonies are apt to run into one another.

After sterilising it is a good plan to allow the tubes to cool in an oblique position, as in this way a larger surface is obtained, and the liquid which separates out collects at the bottom of the tube, and does not interfere with the growth of the cultivation. Glycerin agar is made by the addition of from 5 to 8 per cent. of glycerin after the filtration of the jelly.

Silica jelly.—A special jelly has recently been devised (W. Kühne, 'Zeitsch. f. Biol.,' vol. ix. p. 173) to meet the requirements of some refractory organisms —like those of nitrification—which refuse to grow on gelatine, and demand a medium free from organic matter. In this preparation, which is wholly destitute of organic matter, the gelatinous consistency is obtained by means of dialyzed silicic acid.[1] To one volume of the sterile solution of dialyzed silicic acid placed in a sterile glass dish with flat bottom one-third to one-half

[1] The dialyzed silicic acid is best prepared by taking a solution of sodium or potassium silicate and pouring this into an excess of dilute hydrochloric acid; this mixture is then placed in a dialyzer, and the outside of the latter is kept surrounded with running water during the first day, and subsequently with distilled water, which should be frequently changed until it yields no trace of turbidity with silver nitrate, showing that the whole of the chlorides have been extracted. The contents of the dialyzer, which, if the solution of alkaline silicate originally employed was not too strong, will be quite clear, is then poured into a flask and concentrated by boiling until it is of such a strength that it is found to readily gelatinise on mixing with the saline solution given above. This solution of silicic acid can then be preserved sterile and ready for use in a flask plugged with cotton-wool in the ordinary way. (For further particulars see Winogradsky, *Annales de l'Institut Pasteur*, vol. v. 1891, p. 97.)

volume of a sterile solution of the following composition is added:—

Ammonium sulphate .	·4 gram
Magnesium „ .	·05 „
Potassium phosphate .	·1 „
Calcium chloride .	trace
Distilled water . .	100 grams
Sodium carbonate .	·6–·9 gram

The solution of the sulphates and chloride should be sterilised separately from the solution of the phosphate and carbonate; these two solutions are then preserved in a sterile condition, and equal volumes of each are taken for mixing with the sterile solution of silicic acid.

On thoroughly mixing the silicic and saline solutions gelatinisation takes place in from five to fifteen minutes. The carbonate of soda in the above may often be advantageously replaced by magnesium carbonate, but the medium is then not transparent—a matter which is of no consequence, however, for most purposes for which this jelly is used. The material containing the micro-organisms for cultivation is introduced into and thoroughly distributed throughout the above mixture before gelatinisation has taken place. Or a 'streak culture' may be made on the surface of the jelly after solidification has taken place.

Blood-serum.—The blood of an animal is carefully collected in a sterilised vessel, neglecting a small quantity which first flows from the wound, and is then kept as cool as possible until coagulation commences. When the first few drops of coloured serum make their appearance they are removed with a sterilised pipette. The liquid serum, which subsequently becomes expressed in the course of from twenty-four to thirty-six hours (during which time it must be kept in a refrigerator), is then transferred by means of a sterilised pipette to either sterilised test-tubes, covered glass dishes, or culture flasks, as the case may be. If due care is exercised in these manipulations the serum thus obtained is perfectly sterile. In general, however, it will require sterilisation,

and this is done by exposing the vessels containing the serum to a temperature of 58–60° C. for from one to two hours on five to six consecutive days. For this purpose a water-bath may be used, but special serum sterilisers are more convenient, and may be obtained from any bacteriological apparatus maker. The solidification of the serum is effected at a temperature of from 65–68° C., and can be conveniently carried out in the same apparatus as the sterilisation. The tubes, as in the case of agar-agar, may be sloped, care being taken that the blood-serum is at least an inch distant from the cotton-wool plug. Condensation water collects, as in the case of the agar-agar, and this serves to keep the air within the vessel moist, but if necessary its accumulation can be easily prevented by adding a small quantity (1 per cent.) of gelatine, or 6 to 8 per cent. of glycerin (Nocard and Roux). The latter not only absorbs a considerable proportion of the condensed water, but prevents the formation of a dry scaly surface on the serum. When glycerin is added to the serum it must be heated to between 75° and 78° C. to produce its solidification. Blood-serum prepared in this manner will remain a long time unaltered, but the fresher it is the better are the results obtained. It can, however, be preserved for six to eight weeks without suffering any material deterioration. A thin film of cholesterin forms on the surface of the serum, but this must not be mistaken for bacterial growth.

Blood-serum can be adapted for plate or dish cultures by means of Hueppe's modification. Hueppe uses a mixture of blood-serum and agar-agar by taking sterilised fluid serum at a temperature of 37° C., inoculating it, shaking it in order to ensure the even distribution of the organisms, and then pouring it into a fluid agar-agar meat-peptone solution at 42° C. The mixture is

again well shaken to continue the dispersion of the bacteria throughout the culture fluid, which is then poured on to plates, into dishes or flasks, and allowed to solidify, and then kept at a temperature of about 37° C. Löffler[1] has found that the addition of other materials in small quantities to the blood-serum, while in no way interfering with its subsequent solidification, increases its value as a culture medium. For this purpose meat extract is recommended to which 1 per cent. peptone, 1 per cent. grape sugar, and 0·5 per cent. common salt have been added; the whole of this mixture is boiled up, neutralised with carbonate of soda, and then heated on the water-bath until the albuminates have separated out, after which it is filtered and sterilised in the steamer. This broth is then, on cooling to 50°, thoroughly mixed with the blood-serum, and the whole sterilised by discontinuous heating, and subsequently solidified, as already described. The broth should be added in the proportion of 1 part to every 3 parts of serum.

A number of other variations in the composition of nutritive serum by the addition of different materials may of course be introduced.

Potato cultures.—The common potato forms one of the most valuable and handy of solid culture materials, inasmuch as the majority of micro-organisms grow luxuriantly upon it, and its preparation is a matter of the greatest simplicity. Again, it acts as a restorative to some bacteria which have become weakened; thus the *Bacillus prodigiosus*, so well known on account of the magnificent red pigment which it elaborates, after a time entirely loses the power of producing this characteristic colour, and gives rise only to a dirty grey white substance; but if a portion of such a degenerated growth

[1] *Mittheilungen aus dem kaiserlichen Gesundheitsamte*, vol. ii. 1884, p. 452.

be streaked on to the surface of a potato, the red colour again reappears. There are many ways of preparing potatoes for cultivation purposes, but in all the difficulty of their effectual sterilisation is experienced. The two methods which are mostly used are those in which small circular dishes and test-tubes are respectively employed. The dishes are shallow, being about $\frac{1}{2}$ inch deep, and from $1\frac{1}{2}$ to 4 inches diameter; they are fitted with an overlapping glass cover (see fig. 7). After thoroughly washing and finally rinsing with distilled water, these dishes are placed either in the steam steriliser or in the hot-air oven for a couple of hours. Meanwhile the potato is carefully washed and scrubbed with a nail-brush, and after peeling it is cut into slices which will fit easily into the dish. On replacing the lid the dish with its contents is immediately placed in the steam steriliser, and allowed to remain there for an hour or more; as an additional precaution it can be again sterilised on the following day.

Another method consists in cutting a cylindrical piece out of the peeled potato by means of an ordinary laboratory cork-borer, and placing this in a test-tube with a cotton-wool stopper. One end of the cylinder should be sliced off obliquely, so as to permit of a larger surface being used, as in the case of sloped agar tubes. A drop of water is put at the bottom of the test-tube, to prevent the potato drying up, and the tubes with their contents are then sterilised in the steamer in the usual manner. This is a particularly convenient form of potato culture, as it can be preserved ready for use for a long period of time.

Roux has modified this method, originally devised by Meade Bolton, by constricting the test-tube towards the bottom, as shown in the accompanying figure, so that the condensation water which forms on the walls

of the tube may collect at the bottom without interfering with the potato culture, whilst the latter is thus also furnished with a support upon which it can rest.

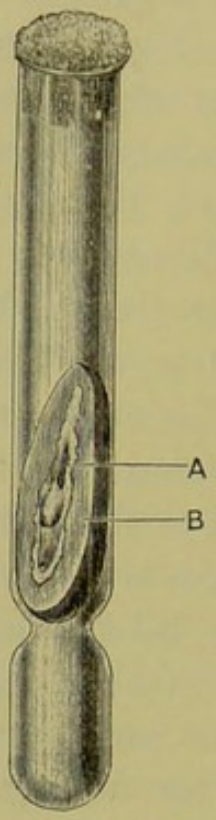


FIG. 4.—ROUX'S METHOD OF CULTIVATING ORGANISMS ON SLICES OF POTATO.

A, growth produced by organism; B, slice of potato.

The same end may be more simply secured by placing a small pad of sterile cotton-wool at the bottom of an ordinary test-tube.

Holz's Potato gelatine.—This medium was devised by Holz[1] originally for the cultivation of the typhoid bacillus, and is prepared in the following manner: Potatoes are carefully washed and peeled, and then pulverised by means of an ordinary kitchen grater. The gratings are collected in glass dishes, and subsequently pressed through a clean cloth, the resulting juice being collected in a flask, which is subsequently plugged with cotton-wool and allowed to stand for twenty-four hours at 10° C. The liquid assumes a dirty brown colour, and after thus standing it is filtered, and the filtrate heated for half an hour in the steam steriliser and again filtered. To 400 grams of this now quite clear potato juice are added forty grams of gelatine, and the whole heated for three-quarters of an hour in the steam steriliser, after which it is filtered and poured into test-tubes. In the test-tubes it is again sterilised for a quarter of an hour on three successive days. This potato gelatine is clear, transparent, and slightly brown in colour. In order to neutralise the material it is necessary to add about 1·6 c.c. of decinormal caustic potash to every ten grams.

[1] *Zeitschrift f. Hygiene*, vol. viii. 1890, p. 143.

Potato cultures used to be considered as capable of assisting very materially in the differentiation of very similar micro-organisms; for example, in many text-books the growth of the typhoid bacillus on potato is given as one of the principal means of distinguishing it from other closely allied forms. Recent research has shown, however, that for such purposes no reliance can be placed on the potato test.

Of great interest and importance in connection with potato culture is a paper by Krannhals,[1] in which investigations are recorded on the growth of Koch's cholera bacillus on potatoes, showing that, contrary to the accepted idea, this organism not only grows on potatoes at 35° C., but that it will also develop at from 15–35° C. on potatoes artificially rendered *alkaline*, or which have spontaneously become alkaline, no growths appearing on acid potatoes at all. Krannhals found that some potatoes which when originally prepared gave an acid reaction, on being tested subsequently exhibited an alkaline reaction, and in this manner he accounts for the impression that the cholera organism will grow on acid potatoes, the change from acidity to alkalinity in the latter having taken place unknown to the investigator. Whether any particular kind of potato is specially liable to exhibit this peculiar behaviour Krannhals is not able to say, but he is of opinion that in all descriptions of growths on potatoes it should be clearly stated whether the medium was acid or alkaline, and that the potato should be tested during the growth of the organism; and, moreover, the appearance of the latter compared with that of its growth on alkalised potatoes. Slices of prepared potato may be rendered alkaline either by saturating them with a 1 to

[1] 'Zur Kenntniss des Wachsthums der Kommabacillen auf Kartoffeln,' *Centralblatt für Bakteriologie*, vol. xiii. 1893, p. 33.

2 per cent. solution of sodium bicarbonate, or by pouring some cubic centimetres of the solution into the culture dish, so that the bottom is only just covered.

In a paper by Kresling[1] the careful selection of the particular potato employed is insisted upon in connection with the growth of the glanders bacillus (*B. mallei*) on this medium. This organism produces acid in solutions containing grape or milk sugar, but not in their absence. This author states that if potatoes which have been frost-bitten or which have begun to bud are used for its cultivation the glanders bacillus produces so much acid that its growth is not only greatly retarded but entirely checked, for the above descriptions of potatoes contain sugar.

Liquid Culture Media

Liquid media have been long used by bacteriologists for cultivation purposes, in fact all the earlier work on micro-organisms was carried out exclusively by their means. Already, in 1857, Pasteur published a memoir on the lactic ferment, in which he employed a transparent culture liquid, and for many purposes these liquid culture media are still preferable, and in some cases quite indispensable. Indeed it may be said generally that whilst the solid media afford great advantages for the preparation of pure cultures and the maintenance of pure growths, the liquid media are of more especial value in the study of the chemical products to which the micro-organisms give rise.

The simplest, although but rarely available, method of obtaining pure cultivations consists in starting a growth of organisms in a liquid medium, which experi-

[1] 'Sur la Préparation et la Composition de la Malléine,' *Archives des Sciences Biologiques publiées par l'Institut Impérial de Médecine Expérimentale à St. Pétersbourg*, vol. i. No. 5, 1892, p. 723.

ence has shown is specially suitable for the particular organism which it is desired to obtain in an isolated condition. When microscopic examination shows that this organism has abundantly multiplied in the medium, a minute quantity is transferred to a fresh portion of the same medium, the growth or multiplication is allowed to take place there, and a small portion is again removed to a fresh quantity of the medium. By repeating this transference a number of times, it is in some cases possible to so purify the growth that finally only one kind of micro-organism is present. The chance of getting pure cultures in this manner is, however, so uncertain that the method generally serves only as a convenient means of preliminary purification. The only reliable way of obtaining pure cultures by means of liquid media is the *dilution method*, an account of which will be found on p. 28.

The following are some of the principal liquid media in use :—

Beef Broth.—The liquid medium which is best adapted for general cultivation purposes is beef broth or bouillon to which an addition of 1 per cent. of peptone has been made. This peptone-beef-broth is prepared in precisely the same manner as has been already described under gelatine-peptone, the omission of the gelatine being the only difference (see p. 19).

Milk.—Milk also affords a good culture material, and may be prepared by simply placing some in sterile test-tubes and steaming it in the steriliser at 100° C. for an hour on the first day, and from 20 to 30 minutes on each of the two following days. By submitting it to such a high temperature, the chemical composition of the milk is altered, however, and in some experiments this would be undesirable. In order to sterilise milk without interfering with its chemical character it is heated

only to a temperature of from 58–65° C. for 1–2 hours on five to eight successive days. At this temperature no coagulation of the albumen takes place, as is the case at higher temperatures, and the milk is at the same time perfectly sterile, and can be preserved for an indefinite length of time. See also p. 5 for the preparation of milk-serum by filtration.

The composition of some of the culture liquids which have been extensively employed in special investigations are now appended:—

Pasteur's Solution.[1]—To 100 parts of water add 10 parts candy sugar, 1 part ammonium tartrate, and the ash of 1 part yeast. Bucholtz substituted for the yeast ash 0·5 grm. of potassium phosphate.

Cohn's Solution.[2]—To 100 c.c. of distilled water add 1·0 grm. ammonium tartrate, ·05 grm. of tricalcium phosphate, ·5 grm. potassium phosphate, ·5 grm. crystallised magnesium sulphate.

Naegeli's Solutions.[3]—(1) To 100 c.c. of water add 1 grm. ammonium tartrate, 0·1 grm. of dipotassium phosphate (K_2HPO_4), 0·02 grm. of magnesium sulphate ($MgSO_4$), 0·01 grm. of calcium chloride ($CaCl_2$). Instead of the ammonium tartrate, ammonium acetate, ammonium lactate, asparagin, or leucin may be added.

(2) To 100 c.c. of water add 1 grm. of egg-albumen peptone or soluble albumen, 0·2 grm. of dipotassium phosphate, 0·04 grm. of magnesium sulphate, 0·02 grm. of calcium chloride.

(3) To 100 c.c. of water add 3 grms. of cane sugar, 1 grm. of ammonium tartrate, and mineral substances as in No. 2.

Percy Frankland's Solutions.—In the study of the

[1] *Annales de Chim. et Phys.* lviii. 323.
[2] *Beiträge zur Biologie d. Pflanzen*, i. 195.
[3] *Untersuchungen über niedere Pilze*, 1882, i.

phenomena of nitrification [1] and for the nutrition of the *nitrous* organism we have employed the following solution :—

Ammonium chloride	·5 gram	In 1,000 c.c. of distilled water.
Potassium phosphate (K_3PO_4)	·1 „	
Magnesium sulphate ($MgSO_4$, $7H_2O$)	·02 „	
Calcium chloride ($CaCl_2$)	·01 „	
Calcium carbonate	5·0 grams	

On the other hand, for the special nutrition of the *nitric* organism Warington [2] has employed

Potassium nitrite	·3 gram	In 1,000 c.c. of distilled water.
Potassium phosphate	·1 „	
Magnesium sulphate	·05 „	
Calcium carbonate	some	

In order to ascertain whether a micro-organism has the power of reducing nitrates, the authors [3] have used a solution of the following composition :—

Potassium phosphate	·1 gram	In 1,000 c.c. of distilled water with 4 grams of pure calcium carbonate in suspension.
Magnesium sulphate (cryst.)	·02 „	
Calcium chloride (fused)	·01 „	
Nitrogen combined in the form of potassium or calcium nitrate	·168 „	
Invert sugar or dextrose	·3 „	
Peptone	·25 „	

On no account must the precaution be neglected of testing the solution for nitrites before use.

In the case of bacteria requiring a more nutritive medium for their growth an addition [4] of potassium nitrate (5 grms. per litre) may be made to broth-peptone and similar culture liquids, but the results obtained are in general not so decisive for diagnostic purposes as when the above weaker solution is employed, whilst the fate of the nitrogen in its various forms is far more difficult to trace.

[1] *Phil. Trans.* clxxxi. (1890) 107.
[2] *Chem. Soc. Journ.* 1891, 519.
[3] *Ibid.* 1888, 374.
[4] Warington, *Chem. Soc. Journ.* 1888, 745.

Uschinsky's[1] *Solutions.*—For the study of the toxic bodies produced, this author has successfully cultivated pathogenic bacteria in the following solution free from albumen :—

Water . . .	1,000 grams	Magnesium sulphate .	·2 gram
Glycerin . .	40–50 „	Dipotassium phosphate	1·0 „
Sodium chloride .	5–7 „	Ammonium lactate .	10·0 grams
Calcium chloride .	·1 gram		

whilst in the following solution he found that they not only grew luxuriantly, but in some cases even more so than in broth :—

Water . . .	1,000 grams	Dipotassium phosphate . . .	2–2·5 grams
Glycerin . .	30–40 „	Ammonium lactate .	6–7 „
Sodium chloride .	5–7 „	Sodium aspartate .	3·4 „
Calcium chloride .	·1 gram		
Magnesium sulphate	·2–·4 „		

Methods for the Isolation of Micro-organisms

Dilution Method.—This method consists in so largely diluting the liquid containing the micro-organisms, and then dividing this diluted material into such a number of small fractions, that each of these fractions contains not more than one micro-organism. Such a fraction then forms the starting-point for a pure culture of the particular organism.

Although the principle underlying this method is obvious enough, and is comprised in these few words, yet its actual execution is in the highest degree laborious and wearisome, success often only being achieved after many abortive attempts. An idea of the manner in which this method is carried out may be gathered from the following hypothetical case :—

Suppose that it has been estimated, by microscopic examination, that about 10,000 microbes are present in one cubic centimetre (about 20 drops) of a given liquid:

[1] *Centralblatt für Bakteriologie*, vol. xiv. 1893, p. 316.

then dilute 1 c.c. to 100 c.c. with sterile liquid, and inoculate 10 tubes each with 1 c.c., each tube will contain about 100 microbes; inoculate 10 tubes each with ·5 c.c., each tube will contain about 50 microbes; inoculate 10 tubes each with ·1 c.c., each tube will contain about 10 microbes. Then dilute 1 c.c. to 1,000 c.c. with sterile liquid, and inoculate 10 tubes each with 1 c.c., each tube will contain about 10 microbes; inoculate 10 tubes each with ·5 c.c., each tube will contain about 5 microbes; inoculate 10 tubes each with ·1 c.c., each tube will contain about 1 microbe; inoculate 10 tubes each with ·05 c.c., each tube will contain about ·5 microbes.

Of the last ten tubes, then, about five only would develop growths, and these would, in all probability, be derived from a single microbe each, and thus be pure cultures.

Now although, in comparison with the gelatine-plate method described below, this dilution process appears tedious and troublesome, yet for the isolation of some micro-organisms it is of the utmost importance, notably in the case of those which, like the bacteria of nitrification, refuse to grow on the ordinary solid culture media. Later on will be found an account of Miquel's application of this dilution process to water-examination.

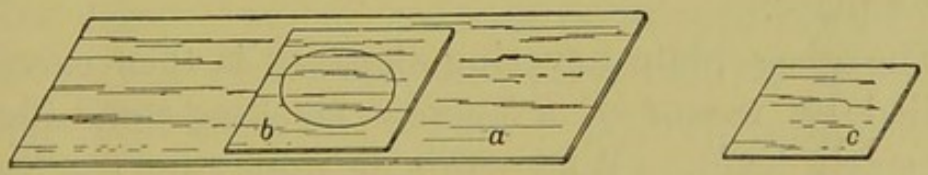


FIG. 5.—HÆMATIMETER (after Jörgensen).

a, Glass slide on which the perforated glass square, *b*, is cemented so as to form an extremely shallow circular cell, the depth of which is accurately determined once and for all. On the glass bottom of this cell some very small squares of known dimensions are etched. A small drop of the liquid in which the number of yeast-cells is to be determined is placed in the cell, and the cover-glass, *c*, placed on the top so as to be in contact with the liquid in the cell. The volume of liquid resting on each of the little squares can thus be easily calculated, and by counting the yeast-cells visible with the microscope in each square, the number in the particular volume of liquid is determined.

Hansen also successfully employed this dilution method in his first preparation of pure cultures of yeast

in 1882, using the *hæmatimeter* to approximately estimate the number of yeast-cells contained in the liquid which was to be diluted.

Gelatine-plate cultures.—Considering, then, what great difficulties attach to the preparation of pure cultures by means of liquid media, it may be imagined how welcome was the introduction by Koch of the new methods of culture on solid media, which greatly facilitated the process of purification.

Ordinary photographic glass plates (quarter-plate size) serve admirably for plate-cultivations. If new, they should be soaked in caustic soda, then washed with water, dilute hydrochloric acid, with water again, and finally rinsed with distilled water. They are then put in a metal box and placed in a hot-air oven and exposed to a temperature of from 150° to 160° C. for two hours. The gas is then turned out and they are allowed to cool, the door of the oven being kept closed from the beginning until the moment when the plates are required for use.

A cylindrical glass dish is filled with ice and water, care being taken that it is quite full, as otherwise in placing the thick glass plate over it bubbles of air become enclosed and thus prevent the uniform cooling of the plate.

The glass plate covering the dish is then carefully levelled by means of a 3-screw levelling-stand (fig. 6) and spirit level. When this is done the condensed moisture which has formed on the surface of the now horizontal glass covering plate is wiped off and a glass bell jar placed upon it. It is convenient to have two such arrangements in use if a large number of plate-cultivations are to be made, a great economy of time being thereby secured.

The sterilised glass plate which is to receive the

gelatine is now carefully withdrawn by means of sterile forceps from the box or oven (the door of which is immediately reclosed), and the future upper surface of the plate is held downwards during its transfer to the levelled plate, and only turned up when the bell jar is momentarily raised to admit it. In this way the falling of air-organisms on the culture plate is avoided.

All is now ready for the gelatine-tubes, which should have been previously melted in a beaker of hot water and then cooled down to 30° C., at which temperature the gelatine remains liquid.

The cotton-wool stopper is first singed in a bunsen-flame to get rid of any chance organisms which may have fallen upon it, and is then removed very carefully, not pulled straight out, but by gently twisting; the mouth of the tube is then passed quickly through the flame to destroy any organisms which may be present, and the contents are poured on to the sterilised glass plate, the bell jar being again lifted for a moment and held over the plate during the operation. After it has congealed, the gelatine-plate is quickly removed to a damp chamber, where it is placed on a glass bench upon which another glass bench can be placed with its gelatine-plate until the chamber is filled.

The damp chamber consists of an ordinary dinner plate covered with a common glass bell jar which fits into the depression of the plate. The air in this chamber is rendered moist by just covering the bottom of the plate with a little sterilised distilled water.

The damp chamber with its contents is allowed to remain for an hour or two in a cold room and is then placed in a cupboard maintained at a uniform temperature of from 18–22° C. The plates prepared as above would remain sterile excepting in so far as they might be accidentally contaminated by aërial microbes

or others gaining access during the operation, and in all experiments it is necessary to pour such plates as a

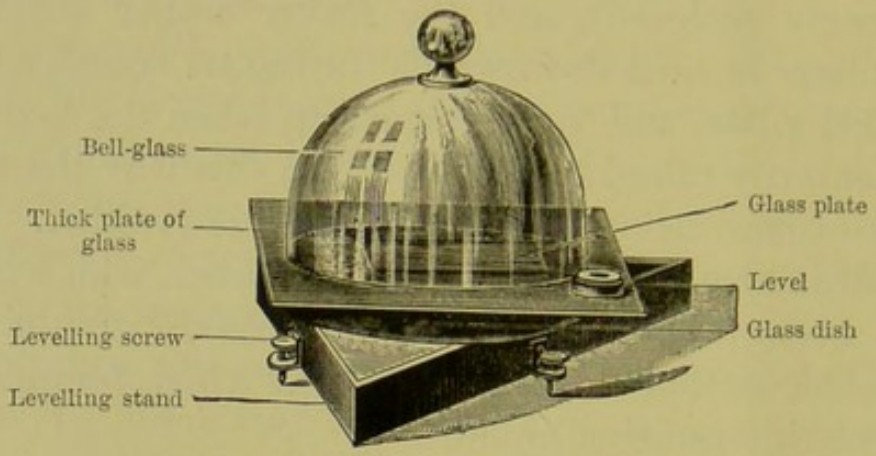


FIG. 6.—LEVELLING APPARATUS FOR MAKING PLATE-CULTIVATIONS.

control for comparison with those which have been purposely infected.

The gelatine-plate method as applied to waters will be described later in detail, but it will be convenient here to give an account of how these plate-cultures are employed for the isolation of particular micro-organisms.

For instance, supposing that we take some bouillon which has been either imperfectly sterilised or purposely exposed to contamination, we should probably find that under the microscope a confused mass of forms would be visible, and to separate out some of these different varieties we abstract a small quantity of the liquid by means of a sterilised instrument. For this purpose a piece of platinum-wire about $\frac{1}{45}$ of an inch in thickness and about three inches in length is commonly used, one end of which is fused into a thin glass rod by melting the latter in a bunsen-flame, inserting the wire, and then allowing the junction to cool slowly. The free end of the wire is then twisted into a small oval loop, which will contain quite a sufficient quantity of the liquid, and the wire being readily sterilised by heating immediately before use in the bunsen-flame, lends itself particularly

well for such manipulations. Care must be taken that the wire is permitted to cool before use, and during the minute which elapses in so doing nothing must be allowed to come in contact with it. It is often quite sufficient to simply use a straight piece of platinum wire instead of bending it into a loop, as in the majority of cases enough material can be conveyed even on the point of such a platinum needle.

Having obtained some of the material, we take a melted gelatine-tube, observing in opening it all the precautions previously described, and insert the infected needle into the gelatine, the needle is then rapidly withdrawn and the cotton-wool stopper replaced. The needle should be immediately sterilised; this is of course especially necessary in working with pathogenic micro-organisms.

The gelatine-tube thus infected must be carefully shaken to ensure the distribution of the micro-organisms throughout the mass of the liquid gelatine; if we were immediately to pour a plate with this we should in all probability obtain such an enormous number of colonies and so densely crowded together as to prevent their proper individual development. To obviate this we take another gelatine-tube and transfer from the original tube, or *first attenuation* as it is generally called, one loop to this second tube, and, after thoroughly mixing, several loops from this second tube to a third. It is essential that in each case the gelatine should be gently but thoroughly shaken, so as to ensure the even distribution of the individual organisms which have been introduced. The amount transferred from one tube to another must be varied according to the judgment of the operator, and after a little practice there is generally little difficulty in procuring successful attenuations or plates in which the colonies are so

distributed that they can develop without any hindrance to each other. If desired all three attenuations can be poured, but it is usually sufficient to take only the second and third. The plates are then incubated at 18–22° C., as previously described, but if a higher temperature be required agar-agar must be employed; but in this event circular glass dishes provided with an overlapping glass cover as described below must be substituted for the plates, as the agar-agar film adheres but feebly to the glass and is very liable to slip off.

Dish method.—The use of culture-dishes[1] is one of the modifications of Koch's process which offers some distinct advantages over the glass-plates. These dishes are about $\frac{1}{4}$ to $\frac{1}{2}$ inch in depth and about $3\frac{1}{2}$ inches in diameter (see fig. 7). They are sterilised in the hot-air oven and are used by partially filling them with sterile gelatine, and then infecting with the living material as was done in the case of the gelatine-tubes above; the cover is replaced as quickly as possible, and the dish gently moved about so as to give the melted gelatine within a rotatory motion and thus ensure the uniform distribution of the micro-organisms.



Fig. 7.—Petri Culture-dish.

A far preferable method of using these dishes, however, is to inoculate a melted gelatine-tube and make from it the several attenuations as usual, then pouring the contents of each gelatine-tube into sterile dishes instead of on to plates as above described. In this manner a much better mixture of the gelatine and the micro-organisms is effected. By the use of such dishes the chance of aërial contamination is reduced, and the

[1] A convenient apparatus for counting the colonies in these dish-cultures has been devised by Lafar, and may be obtained from F. Mollenkopf, 10 Thorstrasse, Stuttgart. Price from 8 to 9 marks.

levelled ice-plate may be dispensed with; in all other respects the details of manipulation are the same as were described for the pouring of the plates.

Tube method.—Another modification of the original method of plate-culture consists in taking rather larger tubes of gelatine, melting the latter and introducing the material for investigation directly into the tube. The cotton-wool plug is then replaced and the gelatine agitated; this must be done gently, to prevent the formation of air-bubbles. An india-rubber cap is then drawn over the cotton-wool stopper, and the tube is carefully rotated in a horizontal position in iced or cold water so as to bring about the solidification of the

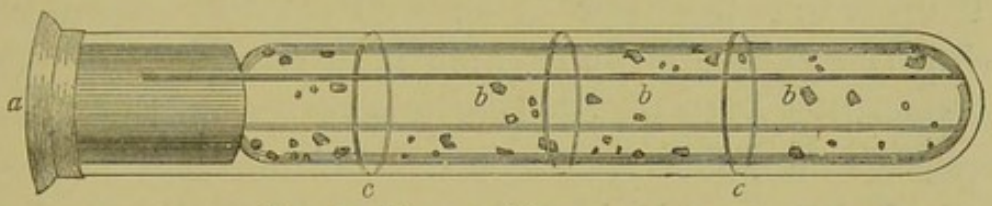


FIG. 8.—ESMARCH TUBE-CULTURE.

a, india-rubber cap; *b*, *b*, *b*, longitudinal line drawn on glass; *c*, *c*, *c*, transverse lines on glass to facilitate the counting of the colonies.

gelatine in an even layer over the internal wall of the tube. On keeping the tube at 18–22° C. the colonies develop in the same manner as in the case of the plates and dishes. This method was devised by Esmarch, and the accompanying figure shows the appearance of such a tube. This method, in consequence of its extreme simplicity, may sometimes be employed with great advantage, whilst a modification of it is most conveniently adapted for the cultivation of anaërobic micro-organisms in colonies (see p. 40).

Whichever of the above methods has been adopted, the colonies, as soon as they have sufficiently developed, are in the first instance examined under the microscope to ascertain what characteristic appearances they present, which will enable them to be subsequently reiden-

tified. A low power, magnifying about 100 times, is all that can be used for this purpose. The plate is removed from the moist chamber and placed on the stage of the microscope, which must be so constructed as to permit of the plate being shifted about that all the colonies can be brought into the microscopic field. Each colony, if it has had space to develop freely, is almost invariably a pure culture, so that by removing a portion of any particular one by means of a sterile platinum-needle to a test-tube containing gelatine or other culture material the growth may be perpetuated in a state of purity. This 'fishing out' of the colonies is easy when there are only a few on the plate, but when numerous and crowded together it is difficult to ensure procuring the particular one which is in request and which can possibly only be distinctly seen under the microscope.

To facilitate this, ingenious contrivances have been introduced by Fodor[1] and Unna.[2]

Of these instruments it is sufficient to say that Fodor's consists of a vertical, adjustable stand (somewhat similar to that of a microscope), carrying a piece into which the glass rod of the platinum-needle can be horizontally clamped; this piece is adjustable in a horizontal plane, and the extremity of the needle is bent vertically downwards for a length of about $\frac{1}{8}$ inch. The plate-culture being placed under the low power of the microscope, and the colony to be removed being visible in the field, the above instrument is approached until the extremity of the needle can be seen with the naked eye to be in the vicinity of the colony in question, but clear of the gelatine-film. The operator then makes the final adjustments with the instrument whilst viewing the colony through the microscope; by depressing the vertical

[1] Fodor, *Centralbl. f. Bakteriologie*, x. 721. [2] Unna, *ibid.* xi. 278.

adjustment of the apparatus the bent-down extremity of the needle is made to dip into the colony, and on raising it again, it emerges with some of the material remaining attached, which can then be further inoculated as desired. The instrument is obtainable from Calderoni & Co. of Deakgasse, Budapest, for 40*s*.

Unna's instrument, which is of simpler and less costly construction, consists of a short metallic tube into one extremity of which a sewing needle can be axially fixed by means of an arrangement similar to that used in pencil-holders, whilst the other end of the tube can be screwed on in place of the microscope lens. The lens after being accurately centred on the colony in question is exchanged for the instrument, and if the latter is properly constructed the needle-point will now be vertically over the colony; on then screwing down the microscope-tube the point of the needle will dip exactly into the colony previously centred with the lens. This 'bacterial harpoon,' as it is called, can be obtained from Zeiss of Jena for 5*s*.

Test-tube inoculations.—A small quantity of the living material to be inoculated is taken on the point of a sterile platinum-needle, and the gelatine-tube into which it is to be introduced is held in the left hand, mouth downwards. The cotton-wool stopper having been previously singed, is now removed, and is held also in the left hand between the third and fourth fingers, care being taken that the part of the stopper which goes inside the tube does not come in contact with the hand or any other object. The platinum-needle is then quickly inserted or stuck into the gelatine and removed, the cotton-wool stopper being then replaced. The tube should be held mouth downwards during the whole operation, so as to minimise the chance of contamination with aërial micro-organisms.

In inoculating from one tube to another, both are held in the left hand in a downward position, the one between the thumb and first finger, the other between the first and second fingers; the stoppers are removed and carefully held between the fingers also of the left hand (one between the third and fourth, the other between the fourth and fifth), and a minute trace of the old cultivation is transferred on the point of the needle to the fresh tube. In the case of agar-agar tubes, in which a certain amount of liquid is present, or in the case of bouillon and other fluid media, the tubes must be held in as inclined a position as possible without allowing any liquid to run up the tubes within reach of the cotton-wool plugs. In inoculating potatoes, the platinum-needle carrying the living material is streaked over the surface of the potato, whilst the cover is very cautiously lifted at one side, if the latter is contained in a dish; whilst in potato tube-cultures the mode of procedure is similar to that already described above for the inoculation of test-tubes.

Anaërobic cultivations.—As some organisms, like the bacilli of tetanus and malignant œdema, have the remarkable property of being unable to grow in the presence of free oxygen, special contrivances have to be introduced for their cultivation and study. A number of devices have been from time to time employed for this purpose, but we shall only describe those which we have found of most general utility.

The following method is admirably adapted for obtaining colonies of anaërobic micro-organisms as well as generally for carrying out experiments on the effect of different gases on bacterial life:—

Larger test-tubes (commonly known as 'boiling-tubes') are charged with about 20 c.c. of gelatine-peptone apiece, plugged with cotton-wool and sterilised

in the usual way. The living material which is to be anaërobically cultivated is introduced on a platinum-needle into the melted gelatine, and thoroughly distributed throughout the latter. The cotton-wool plug is now removed and immediately replaced by an india-rubber stopper, the two perforations in which are fitted with glass tubes, one of which only just opens into the test-tube, whilst the other is long enough to reach nearly to the bottom of the latter. Each of these glass tubes is constricted above the india-rubber stopper, and beyond these constrictions are placed small plugs of sterile cotton-wool; this india-rubber stopper fitted with its glass tubes is sterilised in the steamer, and is not removed from the latter until the moment when required for placing on the test-tube. This having been done, the longer glass tube is connected with a Kipp's or other generator of hydrogen gas, whilst to the outside of the shorter glass tube is attached a piece of narrow india-rubber tubing about one foot in length, which is allowed to hang down loosely. A fairly rapid current of hydrogen is then bubbled through the gelatine, which is kept fluid by immersing the test-tube in a beaker of water at 30° C. The hydrogen escapes through the long piece of india-rubber tubing attached to the shorter glass tube which passes through the india-rubber stopper. When the gas has been passing for ten to fifteen minutes it is stopped, and the two glass tubes are rapidly sealed before the blowpipe at the constricted points referred to above. The india-rubber stopper is then thickly coated with melted paraffin so as to render it perfectly gas-tight, and the tube is rotated in a horizontal position in cold water until the gelatine congeals in a uniform film over the inner walls, as in the ordinary Esmarch-tube (see p. 35). The colonies of those organisms capable of growing in the

absence of air will then make their appearance in due course.

The appearance of the tube with its anaërobic colonies is shown in the figure [1] (fig. 9).

It is sufficiently obvious how the above arrangement can be used for studying the effect of different gases on micro-organisms, whilst a simple modification of the

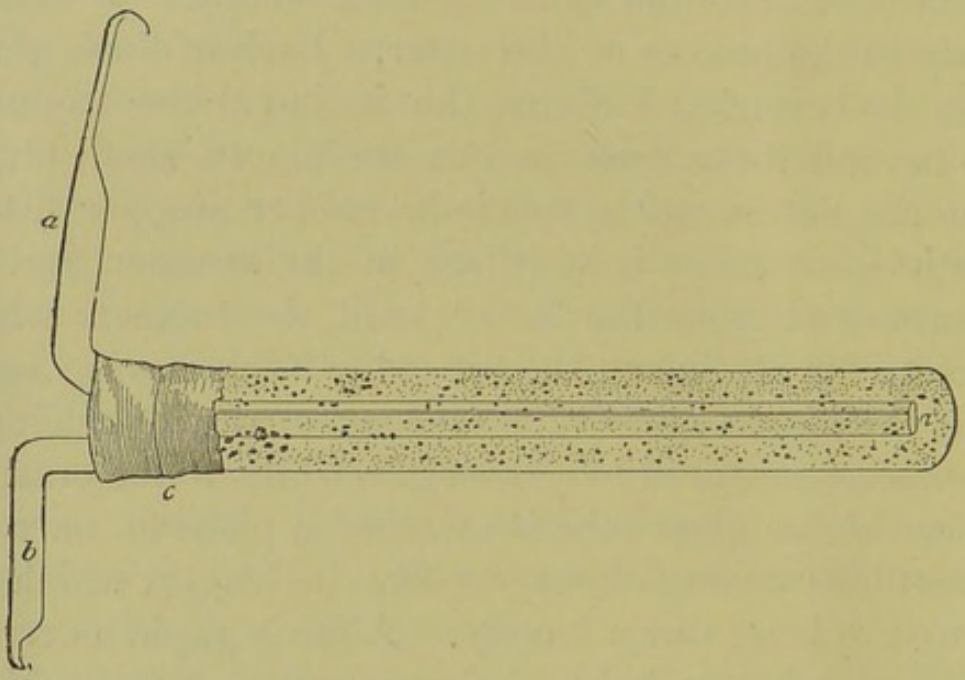


FIG. 9.—C. FRÄNKEL'S ANAËROBIC TUBE-CULTURE.

a, *a*, glass tube through which hydrogen or other gas is passed ; *b*, exit-tube for gas ; *c*, india-rubber stopper, coated externally with paraffin.

ordinary method of plate-culture in damp chambers may also be employed for the same purpose.[2]

Instead of using hydrogen to displace air from a culture material, to be employed for the growth of an anaërobic organism, the removal of the oxygen may be effected by means of bacterial life itself, as devised by Roux, Salomonsen, and Buchner. For this purpose a small culture-tube is fitted in the ordinary way, steri-

[1] Fränkel, *Centralbl. f. Bakteriologie*, iii. (1888) 735, 763.

[2] Percy Frankland, 'On the Influence of Carbonic Anhydride and other Gases on the Development of Micro-organisms,' *Proc. Roy. Soc.* xlv. 292 ; Fränkel, *Zeitsch. f. Hygiene*, v. (1888) 332.

lised and inoculated with the anaërobic organism under examination; it is then placed in a larger tube containing broth which has been infected with *bacillus subtilis*, or some other organism which rapidly consumes oxygen. This outer tube is then tightly closed with an india-rubber stopper, which may be further coated and sealed with paraffin. The oxygen is rapidly removed from the entire closed space by the vegetation of the *bacillus subtilis* in the outer vessel, thus permitting the growth and development of the anaërobic organism in the inner tube.

Instead of using the culture of *bacillus subtilis* in the outer tube, it is more convenient to employ a mixture of caustic potash and pyrogallic acid,[1] which, as is well known, rapidly absorbs oxygen. The arrangement is shown in fig. 10.

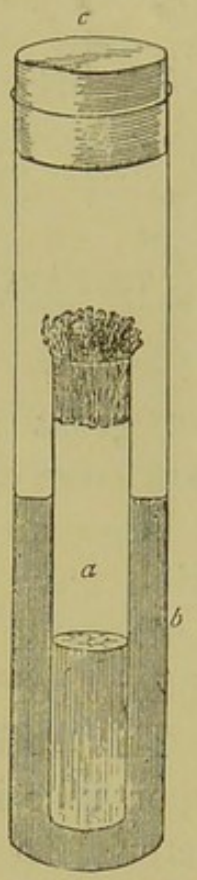


FIG. 10.—ANAËROBIC CULTURE.

a, small test-tube containing culture; *b*, larger test-tube containing pyrogallate of potash solution; *c*, india-rubber stopper.

A useful summary of the various methods which have been devised for anaërobic culture is given by Novy in the 'Centralblatt für Bakteriologie,' vol. xiv., 1893, p. 581.

[1] The solution of caustic potash should consist of equal weights of caustic potash and water, the pyrogallic acid is employed in nearly saturated solution, and one volume of this is mixed with ten volumes of the potash solution at the moment when required.

CHAPTER II

THE STAINING AND MICROSCOPIC EXAMINATION OF MICRO-ORGANISMS

The great advances which have been made in our knowledge of micro-organisms during recent years are in large measure due also to the ingenious methods which have been devised for facilitating their identification and microscopic study by staining them with brilliant colours.

This staining of microscopic specimens is of great importance in many ways. Thus, firstly, it enables the bacteria to be recognised amongst the most varied surroundings in consequence of their particular affinities for some dyestuffs, whereby they may be discovered under conditions in which they would infallibly escape notice if examined in their natural state. In the second place, by the application of these colouring matters far greater precision and definition is given to the forms of the micro-organisms, and by their means even a certain amount of internal structure may in some cases be discerned. Again, inasmuch as the affinities of substances for particular dyestuffs are dependent upon their chemical nature, it is evident that the deportment of micro-organisms towards these colouring matters serves as a micro-chemical reaction by means of which they can not only be distinguished from other bodies with which they may occur in juxtaposition, but which

may actually serve to discriminate between different kinds of micro-organisms themselves.

Even in the dyeing of the textile fibres there is still a very considerable amount of empiricism necessary, and it is not surprising that this should be even more the case in the dyeing of microscopic bodies; the art of successfully staining bacteria under varied conditions is therefore one which can alone be acquired by prolonged practice. In the following pages we have endeavoured to present as concisely as possible the more important methods at present in vogue, arranging them in such a manner as to indicate the relationship subsisting between them, and the particular advantage attaching to each.

Composition and Preparation of various Stains

Of the innumerable dyestuffs which are at present known, practically only the so-called *basic aniline colours* are employed in the staining of bacteria, having been first used for this purpose by Weigert (1875).

These *basic* coal-tar dyes, like magenta, methyl violet, Bismarck brown, exhibit a strong affinity for the protoplasmic contents of bacterial cells as well as for the nuclei of animal tissues, both of which they stain with great intensity.

The *acid* coal-tar colours, like eosine, acid magenta, safranine, picric acid, &c., on the other hand, do not exhibit this special affinity for the nuclei and bacteria, and on being applied, for instance, to a section of animal tissue they stain the latter throughout its entire extent. The natural acid dyestuffs, like hæmatoxylin (logwood) and carmine (cochineal), behave also, on the whole, in much the same way.

These two classes of dyestuffs, the basic and the acid, are therefore sharply distinguished from each

other for the purposes of bacteriological technique, the former being alone available for the more striking exhibition of micro-organisms, whilst the acid colours may sometimes be taken advantage of for the tinctorial demonstration of other elements in the microscopic specimen.

The basic aniline dyes in most frequent use for the staining of bacteria are:—Fuchsine or magenta, gentian violet, methyl violet, methylene blue, Bismarck brown.

It is most convenient in employing these colouring matters to prepare a saturated alcoholic solution of each which can be kept in stock, and these stock solutions are then diluted with about ten times their volume of distilled water for actual use. It is not advisable to prepare more than small quantities of these diluted solutions at a time, as they do not keep their tinctorial powers for very long, although in this respect methylene blue forms an exception, as its diluted solution even is remarkably durable.

Magenta and the two violets possess the strongest tinctorial properties, and are the colours in most constant and general use; but methylene blue, inasmuch as it stains less intensely, is extremely useful in the case of some bacteria, like certain sarcinæ, which take up the colour very strongly, and in which it is sometimes difficult to avoid over-staining the preparations. In fact this stain is particularly useful in revealing the more detailed and delicate structure of micro-organisms.

It has been found that the staining powers of these aqueous alcoholic solutions may be very greatly increased by the addition to them of certain substances, for when used by themselves they are incapable of colouring some bacteria as well as spores and flagella;

hence special devices and methods have to be resorted to in these cases. A summary of the principal of these special stains will now be given. The simplest modification is

Löffler's alkaline methylene blue solution.—To 100 c.c. of a solution of caustic potash (1 : 10,000) add 30 c.c. of a concentrated alcoholic solution of methylene blue. This solution has a much stronger tinctorial power than the simple aqueous solutions of aniline colours, and retains, like all methylene blue solutions, its staining properties if shut up in tightly stoppered bottles for years.

Weigert's solution.—Another simple modification consists in adding ammonia, thus:—To 90·0 grms. of distilled water add 0·5 grm. liq. ammoniæ, 10 grms. of absolute alcohol, and 2·0 grms. of gentian violet.

Ehrlich's solution and modifications.—This solution practically only differs from those we have described above by reason of the alcoholic solution of the basic dyestuff not being diluted with pure water but with water which is saturated with aniline oil. Four to five c.c. of aniline (the well-known oily substance manufactured in such large quantities from the benzene of coal-tar, and which must not be used when it has assumed a brown colour through prolonged exposure to light) are shaken up with 100 c.c. of distilled water, by which the greater portion of the aniline passes into solution. This solution is passed through a *damp* filter so that the excess of undissolved oily aniline particles are retained by the filter, and to the clear filtrate, or 'aniline water' as it is called, are added eleven c.c. of a concentrated alcoholic solution of either fuchsine, gentian violet, or methyl violet. The whole is then frequently shaken during twenty-four hours, at the end of which time the liquid becomes clear

and ready for use. This solution does not keep its staining power for more than from fourteen days to one month.

The above solution has been modified by Löffler and its tinctorial power greatly increased by dissolving 5 grms. of solid fuchsine or any other basic colour in 100 c.c. of 'aniline water' prepared as above. It will retain its tinctorial powers for from four to six weeks, and is best preserved in a tightly stoppered bottle in a dark cupboard. The intensity of the stain may be still further increased by adding a solution (1 : 1,000) of caustic soda drop by drop, until the previously clear coloured liquid just begins to become clouded and before any actual precipitation takes place. This latter modification renders the solution less permanent in its staining properties. In applying this it is best to filter a few drops direct on to the cover-glass preparation which is to be stained.

Another modification of Ehrlich's solution is that introduced by Weigert and Koch. To 100 c.c. of the aniline water add 11 c.c. of a concentrated alcoholic solution of fuchsine or methyl violet and 10 c.c. of absolute alcohol. This solution will keep for from ten to twelve days.

Ziehl's solution.—The principle of this is precisely the same as that of the 'aniline water' solutions just described, the aniline being replaced by the oily substance, carbolic acid. It may be most conveniently thus prepared:—5 grms. of carbolic acid and 1 grm. of fuchsine are added to 100 c.c. of water to which 10 c.c. of alcohol is gradually added. The advantage of this solution is its greater permanence over that of Ehrlich's, although its tinctorial power is not quite so great. Kühne has replaced the fuchsine by adding 1·5 grm. of methylene blue to 10 c.c. of alcohol, which is

mixed, as in Ziehl's solution, with 100 c.c. of a 5 per cent. aqueous solution of carbolic acid.

The decoloration of preparations.—In making microscopic preparations it is often necessary to obtain greater definition by staining in two colours. For this purpose methods have been devised by the use of which, whilst one part of the specimen remains coloured, the other portion is made to give up the stain, after which it is treated with some other colour the application of which does not affect in any way the already stained portion of the preparation. This decoloration is essentially the principle of Gram's well-known method. The strongest decolorising agents are acids. Even a weak solution of acetic acid already exerts a strong decolorising power, whilst weak solutions of hydrochloric, nitric, and sulphuric acid act still more powerfully in this manner. The strongest agents of all for this purpose are acids combined with alcohol. The following are the principal decolorising agents in use :—

(1) 5 per cent. aqueous solution of acetic acid.
(2) 20 per cent. aqueous solution of nitric acid.
(3) 3 per cent. alcoholic solution of hydrochloric acid (100 parts absolute alcohol and 3 parts hydrochloric acid).

Gram's method.—The particular feature of this method, which is of primary importance in the staining of tissues, is that in a section of animal tissue it leaves the micro-organisms stained whilst removing the stain from the animal nuclei. It consists in staining the cover-glass preparation or section in an aniline-water solution of gentian violet for about five minutes, after which it is placed in a solution of iodine and potassium iodide (1 iodine, 2 potassium iodide, 300 parts water) for two minutes, and then washed with alcohol until no more colour is removed; it is then placed in

clove oil, by means of which some more colour is extracted. Gram found, however, that all micro-organisms are not similarly affected by this method of treatment, for whilst some retain, others lose the stain when submitted to the process; the latter may therefore be made use of not merely for distinguishing the micro-organisms from other materials with which they occur, but also as a valuable means of differentiation between various micro-organisms themselves. The method thus acquires a wider importance than that which attaches to the purpose for which it was originally intended.

Gram-Günther method. — Günther has modified Gram's original method by giving the preparation an additional washing with a 3 per cent. alcoholic hydrochloric acid after the alcoholic washing employed by Gram, and also by substituting xylene for clove oil.

The following is the description of this modified method given by Günther himself: [1]—

(1) The section is taken out of the alcohol and immersed in a freshly filtered solution of Ehrlich's aniline water—gentian violet or methyl violet—for from one to two minutes.[2] The solution must have been prepared at least twenty-four hours previously.

(2) The section is removed with a needle, the surplus colour taken off with blotting-paper and placed in the solution of iodine and potassium iodide [3] for two minutes. The section should lie well spread out on the bottom of the dish.

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[1] *Einführung in das Studium der Bakteriologie mit besonderer Berücksichtigung der mikroskopischen Technik.* Leipzig, 1893. P. 102. 3rd edition.

[2] With tubercle and leprous sections the time is longer; in the case of the former from twelve to twenty-four hours are required, whilst in the latter half an hour is sufficient.

[3] Botkin advises, before treating the preparation with the iodine and potassium iodide solution, to wash it well with pure aniline water to remove the surplus colour, and then proceed as above. He states that the colour is thus more easily removed, and the preparation can remain for a much longer time in the iodine and potassium iodide without suffering any damage—that in fact he has obtained cleaner and better stained

(3) Placed in alcohol for half a minute.

(4) For ten seconds in 3 per cent. alcoholic hydrochloric acid.

(5) At the end of ten seconds it is at once immersed in fresh pure alcohol for several minutes.

(6) After being transferred once or several times to fresh alcohol in order to remove the maximum amount of colour it is placed in xylene.

(7) In xylene the section may remain for any length of time, as it undergoes no change in it. After being saturated with xylene for not less than half a minute, it may be placed on the microscopic slide.

(8) After soaking up the excess of xylene a drop of xylene balsam is placed on it and the cover-glass superposed.

If it is desired to colour the nuclei of the tissue as well as the bacteria, and so stain the preparation in two colours, it is advisable to stain the nuclei *before* the bacteria; this can be done as follows:—The freshly prepared unstained sections are taken out of the alcohol, and instead of proceeding as above they are placed for several minutes in water, and then in a picrocarmine solution (picrocarmine does not stain bacteria) for from one to two minutes. (The picrocarmine solution is thus prepared:—To 50 parts of distilled water add 1 of carmine and 1 of ammonia; to this add a solution of concentrated aqueous picric acid until no more carmine is precipitated.) The section is washed four or five times in water and then placed in alcohol. The cell-nuclei are now beautifully stained with carmine, and the section may remain unharmed for a convenient time in the alcohol, after which it can be stained according to the Gram-Günther method, proceeding exactly as if it were a colourless preparation, the cell-nuclei retaining their tint without the least alteration during the process.

It is very important to note that fuchsine, methylene blue, and Bismarck brown cannot be used for Gram's method, but only the so-called *pararosaniline*

specimens by adopting this precaution.—*Centralblatt für Bakteriologie*, vol. xi. p. 231, 1892.

colours (Unna [1]), to which belong methyl violet, gentian violet, and Victoria blue, the strong affinity of these for iodine being, according to Unna, the cause of this remarkable circumstance.

Double staining is also resorted to in the case of the spores of micro-organisms, which will not take up the colouring matter in the usual way. Special methods have to be devised for the exhibition of both these and the organs of locomotion, or flagella, with which some bacteria are provided; these will be described later.

Cover-glass preparations of micro-organisms.—Having described the various stains which are in use for the colouring of micro-organisms, we must now consider the means by which such stained preparations may be microscopically examined. In the first place, the cover-glasses must be scrupulously clean, every trace of grease being removed. To accomplish this successfully they may be heated for some minutes in concentrated sulphuric acid, then washed with distilled water and transferred to a mixture containing equal parts of alcohol and ammonia, after which they should be dried with a perfectly clean soft rag. The cover-glass may finally, before use, be passed a few times slowly through the flame of a bunsen, so as to effectually remove every particle of grease. The glass slides on which the cover-glasses are mounted should also be thoroughly cleaned and freed from dust.

As it is important in examining cultivations of micro-organisms that there should not be too much material on the cover-glass, it is best to mix a small quantity abstracted on the point of a sterile platinum-needle, with a drop or two of distilled water on a cover-glass, and from this dilution to remove some by means

[1] *Die Rosaniline und Pararosaniline.* Dermatologische Studien, 4tes Heft. Hamburg and Leipzig, 1887.

of a looped needle to another cover-glass, and spread it as evenly and thinly as possible over the surface. Care must be taken that practically none of the culture material is introduced along with the organisms, as this spoils the preparation, rendering it indistinct and dirty.

When the cover-glass with its thin film of material has become perfectly air-dry,[1] but not before, it should be taken up with a pair of forceps at one corner and heated in a spirit flame or bunsen-burner. This heating of the preparations is a matter of great importance, for unless the material is treated in this manner it will not adhere to the cover-glass, and when subsequently rinsed with water it will be washed away. The degree of heating is also a matter requiring great care, for should the preparation be over-heated the bacteria will not take up the stain satisfactorily, whilst the albuminous matters in insufficiently heated specimens often give rise to precipitations on adding the stain. Experience, however, has shown that, as a general rule, by passing the cover-glass without stopping three times in succession vertically through the flame of a bunsen-burner, the requisite degree of heating is secured. Of course in this, as in all other manipulations, only practice will enable the student to attain the judgment necessary for carrying out successfully such details. When the preparation is thus *fixed* it is held with the forceps in the left hand, whilst with the right a few drops of stain are poured on to the cover-glass by means of a narrow glass pipette; the stain must be evenly distributed, and entirely coat the surface of the cover-glass. After from one to ten minutes, according to the strength of the stain and the nature of the organism, the cover-glass

[1] This air-drying may be accelerated by means of a desiccator, or by a rapid current of air directed on to the cover-glass with a spray-producer, or by *very gentle* heat, but the latter requires the greatest caution.

should be thoroughly rinsed with distilled water by means of a wash bottle, and is then turned preparation-side downwards on to a clean slide, gently pressed with blotting-paper, so that all moisture on the upper surface is removed. It is now ready for examination with the oil immersion lens, a drop of cedar oil being first placed on the dried surface. This is only a very temporary though rapid way of mounting preparations. If they are required for permanent use—as a reference, for example—they must be mounted in canada balsam. In this case the cover-glass must, after staining and washing, be allowed to become quite dry; a small drop of balsam is placed on the glass slide, and the cover-glass is then, preparation-side downwards, deposited on the centre of this drop of balsam, which spreads out, and finally extends over the whole under-surface of the cover-glass. After a few days the balsam has become hard, and after a few weeks extremely hard at the edges. This is called a permanent preparation, and the colour will remain for a long time unchanged if it is preserved in the dark.

Staining of spores.—If a preparation of, say, *b. anthracis* or *b. subtilis* containing bacilli and spores be stained in the usual way with the ordinary aqueous solutions of aniline colours, bright spots will be found, sometimes in the middle or at one end of the stained bacilli, or in isolated groups, which have not taken up the colouring matter. These spots are the spores which, as in the case of some refractory bacilli, the stain has been unable to touch, but which by special treatment may also be beautifully exhibited by coloration.[1] For

[1] Not all such unstained spots apparent sometimes in coloured preparations are necessarily spores. Often when the culture is old and degeneration of the bacilli has taken place, clear spaces which have not taken up the stain are seen in the middle of the rod. Other causes may also contribute to this appearance.

this purpose it is necessary to use a stronger dye and to employ heat to assist its power of penetrating the spore. Ehrlich's fuchsine solution (see p. 45) is best adapted for this purpose, and after the cover-glass has been treated in the usual way with the stain, it is held over a small flame, care being taken to move it backwards and forwards the whole time. The cover-glass must be removed directly it begins to steam, and on no account must the liquid be allowed to boil, as this will spoil the preparation. The cover-glass is then washed in the ordinary way and is ready for examination. The spores will be found to have now assumed a strong red colour, and by the greater intensity of their tint may be distinguished from the bacilli. This, however, does not exhibit the spores so perfectly as when the bacilli are stained a different colour. For this purpose recourse is had to a method of *double-staining*, as described below.

Double-staining.—The cover-glass stained as above is treated with a decolorising agent, being washed with a 5 per cent. solution of acetic acid until experience indicates that the bacilli will have lost all their colour, whilst the spores, which both take up and part with the dye less readily, will remain tinted, although less intensely than before. The preparation is then thoroughly washed with water and treated with the ordinary aqueous solution of methylene blue. The spores, which are not affected by the latter aqueous stain, will still remain red whilst the bacilli have assumed the blue colour.

The above process may be modified in detail as recommended by Günther, thus: instead of heating the cover-glass preparation in the flame, it is placed preparation-side downwards in a watchglass filled close to the brim with the freshly prepared Ehrlich's solution. The solution with the cover-glass is now gently heated over

a very small flame, care being taken to move it up and down in a vertical direction above the flame until bubbles begin to appear on the surface; it is then allowed to cool for one minute, when it is again heated as before until bubbles rise, and then subsequently cooled. This is repeated about five times. The cover-glass is then removed from the hot solution and placed in a dish with a 3 per cent. solution of alcoholic hydrochloric acid (100 parts of absolute alcohol to three of hydrochloric acid), the preparation-side of the cover-glass upwards. It should remain in this solution for one minute, and is then washed with water as usual and coloured with methylene blue. If gentian violet is used instead of fuchsine, the bacilli must be stained after the decoloration with Bismarck brown.

Fiocca[1] recommends the following method for staining spores, which he states is not only very successful, but very expeditious: About 20 c.c. of a 10 per cent. ammonia solution is poured into a dish, to which is added ten to twenty drops of an alcoholic solution of the aniline dye employed. The whole is heated until steam begins to rise, when the ordinary cover-glass preparations are introduced. On an average three to five minutes' immersion is sufficient; in the case of very obstinate spores, such as those of anthrax, ten to fifteen minutes is necessary. The cover-glass is removed and quickly placed in a decolorising solution, such as a 20 per cent. solution of either sulphuric acid or nitric acid. The preparation is then washed with water and stained with an aqueous solution of some colour in contrast to that used for the spores.

Staining of flagella.—The flagella, or organs of locomotion, which are attached to some bacilli and

[1] 'Ueber eine neue Methode der Sporenfärbung,' *Centralblatt für Bakteriologie*, vol. xiv., 1893, p. 8.

even to some micrococci, offer yet greater obstacles to exhibition by staining than do the spores. In fact, to reveal the presence of these delicate thread-like appendages by coloration, it is necessary to make use of what is known to dyers as a *mordant*. The application of this mordant or biting material enables the flagella to subsequently fix the dye, for which they have otherwise no affinity. It is to Löffler [1] that we are indebted for the developments in microscopical technique which enable the flagella to be so easily and beautifully exhibited in stained specimens. Löffler has further found that in mordanting some varieties of micro-organisms an acid, and in others an alkaline, addition to the mordant must be made, and moreover that the exact amount required varies in both cases according to the particular organism under investigation. To render the mordant alkaline Löffler recommends the use of a 1 per cent. aqueous solution of sodium hydrate, whilst for the acidification of the mordant he employs dilute sulphuric acid of such strength that a given volume is exactly neutralised by the same volume of the 1 per cent. solution of caustic soda.

The following is the composition of the mordant:—

Solution of tannin (20 parts tannin + 80 parts water).
To 10 c.c. of this tannin solution add
5 c.c. of a cold saturated solution of ferrous sulphate and
1 c.c. of a concentrated solution, either aqueous or alcoholic, of fuchsine.

For many organisms the simple treatment with the mordant is sufficient, but in the case of others, as before mentioned, an acid or alkaline addition is requisite.

After the preparation has been mordanted with the above solution it is dyed as usual with the 'aniline-water' solution of fuchsine previously described. The

[1] *Centralblatt f. Bakteriologie*, vol. vi., 1889, p. 209; also *Ibid.* vol. vii., 1890, p. 625.

exact manner in which the whole process is carried out is as follows :—

The cover-glasses must be perfectly clean, and especially free from all grease (see p. 50), and no trace of the culture material must be introduced with the organism. Care must be taken that the culture is young and that only a very small number of organisms are abstracted, as if the preparation is too crowded it effectually prevents the flagella from being seen. Great care must also be taken not to overheat the cover-glass with the air-dried specimen in passing it through the flame (see p. 51); it is best, in fact, to hold the cover-glass between the finger and thumb instead of using forceps for this operation, as the temperature which is endured by the finger does not injure, but is also quite sufficient for the purpose. The mordant is then run from a pipette on to the cover-glass, and the latter is held over a flame and gently moved up and down the while until the liquid begins slightly to steam. This heating must only last for half to one minute; the liquid is then poured off, and the cover-glass most thoroughly washed with water; it is then allowed to dry in the ordinary way, and a few drops of 'aniline-water' fuchsine solution poured on to it; it is then gently warmed for about a minute in the flame, after which the stain is washed off very thoroughly with water and the cover-glass is ready for examination.

The following are the additions of acid and alkali respectively made to the mordant as recommended by Löffler for particular micro-organisms :—

(22 drops = 1 c.c.)

Spirillum choleræ asiaticæ	.	1 drop	of acid	to 16 c.c.	of mordant
„ rubrum (Esmarch)	.	9 drops	„	„	„
„ Metchnikoffii	.	4 „	„	„	„
Bacillus pyocyaneus	.	5 „	„	„	„
Spirillum concentricum	.	0 „	„	„	„

Bacillus mesentericus vulgatus	4 drops of caustic soda to 16 c.c. of mordant				
Micrococcus agilis . . .	20	„	„	„	„
Typhoid bacillus . . .	22	„	„	„	„
Bacillus subtilis . .	28 to 30	„	„	„	„
„ œdematis maligni .	36	„	„	„	„
„ of symptomatic anthrax	35	„	„	„	„

Löffler's method not only stains the flagella, but the whole cell. In the ordinary processes of staining with basic colours only the protoplasmic body of the micro-organism is coloured, the outer covering but rarely taking up any dye at all; this process, however, colours both the cell-wall and the protoplasmic contents, so that when stained in this manner the bacteria look thicker than when dyed in the ordinary way.

A simple modification of the above method has been more recently devised and successfully employed by Nicolle and Morax.[1] This consists in taking a small quantity of a recent agar-culture of the organism and diluting it in sterilised ordinary water in a watch-glass; the liquid should be only very slightly turbid. A small portion is then run on to cover-glasses, which must be scrupulously clean and free from grease, for which purpose it is advisable to first heat them thoroughly by passing them several times through a bunsen-flame. The cover-glass is held with forceps at one corner, and after the liquid has spread over the surface the glass should be slightly inclined, and the excess of liquid which gathers at the opposite corner removed by aspiration through a pipette, and the surface allowed to dry, but protected from dust. A large drop of the mordant or fuchsine-ink, prepared according to Löffler's recipe,[2] is then run on to the cover-glass,

[1] 'Technique de la Coloration des Cils,' *Annales de l'Institut Pasteur*, vol. vii., 1893, p. 554.

[2] These authors mention particularly that the tannin must be of the best quality.

which is heated for about ten seconds over a small flame until it begins to steam; the mordant is then shaken off, and the cover-glass inclined and washed in a stream of distilled water, care being taken that the preparation is not detached. The mordant is then run on again, heated, and the cover-glass washed as before, which process should be repeated altogether three or four times. The under-surface of the cover-glass as well as the points of the forceps must be carefully dried after each washing, otherwise on subsequently applying the mordant it will extend on to the under-side of the glass as well as on to the forceps. The staining is very simple, and may be effected by applying Ziehl's fuchsine solution to the surface of the preparation, and heating it once or twice for a quarter of a minute, or by using even the ordinary aqueous solutions of violet. After the stain has been washed off in water the preparation is ready, and may be examined in the usual way under the microscope. Thus the addition of an acid or alkali is omitted, these authors stating that it is not only tedious, but does not serve any useful purpose, equally good results being obtained by applying the mordant three or four times, instead of only once as recommended by Löffler.

Drop-cultures of Micro-organisms

The most convenient mode of studying micro-organisms in the living state under the microscope, *e.g.* in order to ascertain whether they are possessed of motility or not, is in what is known as *drop-culture*. An excavated glass slide is, after careful cleaning, sterilised by heating in a bunsen-flame; a little vaseline is then applied round the cup, and it is placed under a glass bell jar. It is best to hold the slide with the excavated side downwards when sterilising it in the flame, and to keep it in this position

whilst applying the vaseline, so as to avoid any contamination through air-organisms falling on to the surface. A cover-glass is held with forceps, and sterilised by means of the flame, and when cool a drop of bouillon is placed on the centre of its under-surface, either by means of a platinum loop or a sterilised pipette. The merest trace of material containing the desired organism is then conveyed on the point of a platinum-needle and introduced into the broth. The cover-glass is now placed, with the drop of infected bouillon downwards, on to the excavated slide, so that the drop hangs down into the middle of the depression in the slide. The vaseline forms a seal from the outer air, which may be further increased by another touch with vaseline all round the edges of the cover-glass. Of course no vaseline must be allowed to get on to the surface of the cover-glass, where the oil will be subsequently placed prior to examination with the immersion lens.

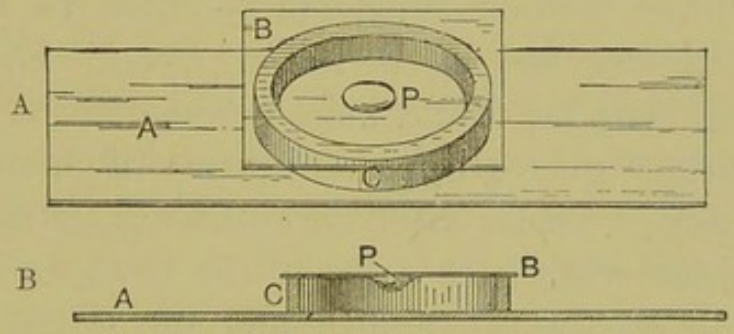


FIG. 11.—SUSPENDED DROP-CULTURES.

FIG. A.—A, glass slide; B, cover-glass; C, glass ring forming the wall of the chamber; P, drop of nutritive liquid in which the micro-organisms grow (Klein).

FIG. B.—Side view of above.

Drop-cultures thus prepared will last for a long time, and may be studied from hour to hour or day to day.

Instead of an excavated glass slide, a small glass ring may be cemented on to a plane slide, so as to form a little cell in which the drop-culture hangs, as shown in the figures.

CHAPTER III

THE EXAMINATION OF WATER FOR MICRO-ORGANISMS

Collection of samples.—Samples of water for bacteriological examination may be collected in flasks plugged with cotton-wool,[1] in glass bottles closed with tightly fitting glass stoppers, or in sealed flasks from which the air has been exhausted, and the necks of which have been drawn out to a fine point. This fine point is broken off under water, and after the latter has rushed in to fill the vacuum, the flask is sealed up again on the spot in the flame of a spirit lamp.

All these vessels must of course be carefully sterilised before use in the hot-air oven. For general purposes it will be found most convenient to use small wide-mouthed glass-stoppered bottles of about 60–100 c.c. capacity. These, after careful washing and rinsing with distilled water, are dried in the oven, each stopper being laid across the mouth and not fitted into the neck of the bottle to which it belongs. When quite dry the stoppers are tightly inserted, and each bottle is shut up in a separate small tin canister; these canisters with their enclosed bottles are then heated for two to three hours at 150° C. in the oven. The bottles are transported in their canisters to the place where the water is to be collected, and not until the moment

[1] In the case of samples which have to be transported any considerable distance before reaching the laboratory cotton-wool stoppers are not advisable.

of taking the sample is the bottle withdrawn from its tin.

The following instructions for the collection of samples of water for bacteriological examination should be as closely as possible adhered to :—

1. The canister containing the sterilised bottle should not be opened until everything is ready for the collection of the sample.

2. After opening the lid of the canister, the sterilised bottle is to be lifted out by its stopper, which is seized with forceps previously heated in a flame, the bottom of the bottle is held with the fingers of the left hand, and the stopper is then to be screwed off by means of the forceps which are held in the right hand, and on no account must either stopper or mouth of the bottle be allowed to come in contact with the hand or any other object. The open mouth of the bottle is then placed under the tap or spout from which the sample is to be collected, the water being allowed to flow in until the bottle is almost but not quite full. The stopper, which in the meantime has been carefully held with the forceps *by its handle*, and not laid down anywhere, is at once replaced and tightly screwed in, the bottle being lifted back into its tin by the stopper and the lid closed. The tin should be so placed that the bottle remains upright.

In collecting samples from cisterns, tanks, ponds, rivers, or lakes, it is best to completely immerse the bottle in the water before withdrawing the stopper, and to replace the latter before again raising the bottle above the surface. In this manner any surface scum which may be present on the water is avoided, but at the same time in cases where the water is shallow, as in some streams, any disturbance of the sediment must be carefully prevented.

If the water is to be collected from a tap (filter or other), the latter should be allowed to run for five or ten minutes before the sample is collected. When samples of well water are required care must be taken that the water has been pumped for some ten or twenty minutes previously, in fact to obtain a representative sample pumping operations should have been going on continuously for several hours, or even days, previous to its collection. (See chapter on Bacterial Contents of Various Waters, p. 102.)

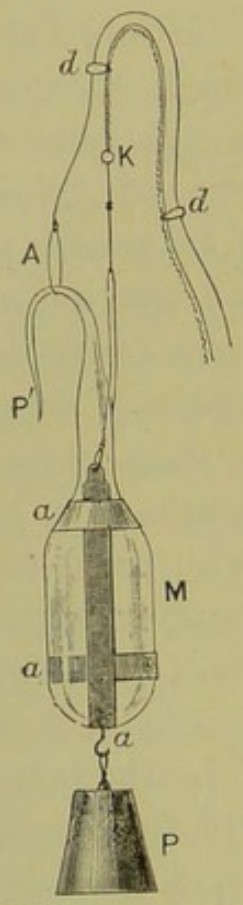


FIG. 12.—APPARATUS FOR COLLECTING SAMPLES OF WATER AT DEFINITE DEPTHS (Miquel).

For the collection of samples at considerable or at specified depths, the following apparatus devised by Miquel may be found of service :—

It consists of a glass vessel M, of about 50 c.c. capacity, the neck of which is drawn out to a fine point and bent as seen at P′, held in its place by metal bands *a a a*, to which a weight P from 2 to 3 kilograms is attached. The whole is suspended by means of a strong cord graduated in metres, yards, or feet by means of rings and knots. Running alongside of this cord a thread of copper passes through the rings *d d*, situated a metre or yard apart. Attached to the end of the copper thread is a ring A, which encloses the fragile neck of the vessel. When the apparatus has been lowered to the required depth, the neck is broken off by giving a quick, sharp pull to the copper wire, and the water rushes into the sterilised and vacuous vessel. It is then drawn up, and the depth carefully noted to which it has been lowered. If the water is not to be immediately examined,

the broken neck of the vessel must of course be sealed in a flame.

In all cases as short an interval as possible should elapse between the collection of the sample and its subsequent examination, and during any such interval the water should be placed in as cool a place as possible, or it may be packed in a box surrounded with ice and so forwarded to its destination. The arrival of a sample when sent by train should be advised beforehand if possible, so that any delay in its delivery may be avoided. It is, however, far preferable for the operator to make the cultivations on the spot, and thus avoid all risk of multiplication during transit.

Special culture media employed.—In order to ascertain the bacterial contents of any given water for general purposes, it is obviously of importance that those culture media permitting of the development of the largest number of micro-organisms should be employed. In the case of those waters in which special points require investigation, *e.g.* in examining for the presence of the typhoid bacillus, &c., we shall indicate later on (see p. 267) what are the particular methods in use; we shall also have to consider the bacteriological examination of waters used for brewing purposes, and the special modifications which are then adopted. But it will be convenient, in the first instance, to point out certain modifications in the composition of the ordinary gelatine-peptone medium (the preparation of which has been described on p. 9) which have been found to influence the number of colonies making their appearance on the plate-cultivations of waters.

Some interesting experiments have been made in this connection by Reinsch,[1] on the addition of different

[1] 'Zur bakteriologischen Untersuchung des Trinkwassers,' *Centralblatt für Bakteriologie*, vol. x., 1891, p. 415.

quantities of a concentrated solution of sodium carbonate to the gelatine-peptone, and the effect produced thereby on the development of water-microbes. The water selected for experiment was abstracted from the Elbe below Hamburg and Altona. As this water contains a very large number of microbes it was diluted with distilled water, and the figures given below have reference to this diluted water. The following table shows the results obtained :—

Quantity of sodium carbonate added to 10 c.c. of ordinary slightly alkaline gelatine-peptone	Number of colonies obtained per ½ c.c. of water
Without any addition	475
0·00504 gram sodium carbonate	1,140
0·01008 „ „ „	2,976
0·02016 „ „ „	2,486
0·03024 „ „ „	1,612
0·05040 „ „ „	1,302
0·07560 „ „ „	748
0·10080 „ „ „	348
0·15120 „ „ „	216
0·20160 „ „ „	74
0·30240 „ „ „	0

This table shows clearly the effect on the development of the water bacteria of the addition of this alkali, the introduction of 0·01 gram sodium carbonate causing the number of colonies found to be six times as great as that revealed by using the ordinary gelatine. Reinsch has also experimented on the effect of adding varying quantities of acid to the ordinary gelatine, and his results are recorded in the following table :—

Quantity of tartaric acid added to 10 c.c. of ordinary slightly alkaline gelatine-peptone	Number of colonies obtained per ½ c.c. of water
Without any addition	406
0·0028 gram tartaric acid	340
0·0056 „ „ „	173
0·0084 „ „ „	19
0·0112 „ „ „	11
0·0140 „ „ „	6
0·0224 „ „ „	0

Thus with the increasing acidity of the medium the number of colonies developed steadily diminishes.

These experiments show how important it is that in *comparative* examinations of water by different observers, the alkalinity of the gelatine-peptone employed should be most carefully determined. Reinsch points out how unsatisfactory for such purposes is the estimation of the degree of alkalinity of the culture material by litmus-paper, as the sensitiveness of the latter is so exceedingly variable. In order to accurately determine the alkalinity of any given culture material, this author suggests the adoption of Schultz's [1] method. Schultz recommends the addition of a 0·4 per cent. solution of caustic soda, which is carefully measured out in drops by means of a burette. Thus 1 c.c. of the culture medium is placed in a test-tube, to which a drop of phenolphthaleïn is added as indicator; caustic soda is then run in until the liquid becomes of a faint pink colour.[2] (The vessel containing the culture liquid should stand on white paper so that the colour may be more easily recognised.) For the sake of accuracy this should be repeated three times, and the mean taken of the three observations, in order to ascertain the amount of caustic soda which should be added to the main body of the culture material in order to secure the particular degree of alkalinity required.

To calculate the amount for a larger quantity of the culture medium it is only necessary to multiply the quantity of the alkali required for 1 c.c. by the number of cubic centimetres of culture material to be neutral-

[1] 'Zur Frage von der Bereitung einiger Nährsubstrate,' *Centralblatt für Bakteriologie*, vol. x., 1891, p. 52.

[2] This pink colour, which is produced in the presence of the phenolphthaleïn, indicates that the liquid contains free alkali, that in fact its reaction is now alkaline. Of course the phenolphthaleïn is not added when the neutralisation of the larger quantity of material is effected, but only in the test-titration.

ised. But as the neutralisation of the larger quantity of material is effected by a stronger (4 per cent.) solution of caustic soda, it is necessary, after multiplying the amount of the 0·4 per cent. solution required for so many cubic centimetres of material, to divide the result by 10. For example, suppose 1 c.c. of broth requires 0·25 c.c. of a 0·4 per cent. solution for its neutralisation, 1,000 c.c. will require 250 c.c. of the 0·4 per cent. solution, or 25 c.c. of the 4 per cent. solution. By adopting this method the degree of alkalinity can of course be far more carefully regulated and accurately determined than by the litmus-paper test, and it should be resorted to in those cases in which special attention is to be directed to the numerical estimation of colonies. On the other hand, in those numerous cases in which comparative results only are desired—*e.g.* in the examination of a water before and after filtration or other treatment—the precise degree of alkalinity of the medium is of less importance.

Dahmen[1] has more recently investigated the effect on the development of water bacteria of the degree of alkalinity possessed by the culture medium employed. Sodium carbonate was added in varying quantities to the gelatine-peptone, and the result traced in the development of the bacteria present in water obtained from the Rhine. It was ascertained that the addition of 0·15 per cent. sodium carbonate induced the development of the largest numbers of water microbes. These results confirm those previously obtained by Reinsch in the case of the river Elbe water.

Dahmen[2] has also conducted experiments on the

[1] 'Die bakteriologische Wasseruntersuchung,' *Chemiker-Zeitung*, Jahrgang xvi., 1892, No. 49.

[2] 'Die Nährgelatine als Ursache des negativen Befundes bei Untersuchung der Faeces auf Cholera-Bacillen,' *Centralblatt für Bakteriologie*, vol. xii., 1892, p. 620.

addition of soda crystals to ordinary gelatine-peptone, with special reference to the development of the cholera organism. It was found that slightly alkaline gelatine, as usually recommended, was not suitable for the cultivation of the comma bacillus, but that gelatine to which 1 per cent. of soda crystals had been added gave excellent results, and greatly facilitated the detection of the bacillus in fæces in the presence of other organisms. Aufrecht ('Centralblatt f. Bakt.' vol. xiii. p. 354) confirms these observations on the favourable results obtainable by this addition. (In this connection see also p. 276.)

Pohl[1] has also introduced modifications in the composition of the gelatine-peptone which he employed in the examination of some marsh-water, whereby he was able to isolate four new varieties, which whilst growing luxuriantly in the modified medium hardly developed at all, and were quickly crowded out by the other water bacteria, in the ordinary gelatine-peptone. These successful results were obtained by adding ammonium carbonate to the culture medium in the proportion of from 0·5 to 1 per cent. Pohl recommends that the preparation of this ammonia-gelatine should be carried out in the following manner:—The gelatine-peptone is sterilised as usual, and mixed with a carefully sterilised solution of ammonium carbonate; this mixture may be subsequently heated for half an hour in the water bath to ensure absolute sterility, but if it is heated for longer than this the greater part of the ammonium carbonate is lost, and the gelatine moreover, being overheated, solidifies less readily.

The bacteriological examination of brewing waters

[1] 'Ueber Kultur und Eigenschaften einiger Sumpfwasser-Bacillen und über die Anwendung alkalischer Nährgelatine,' *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 141.

(*Hansen*).—In the case of water used for brewing purposes, the mere numerical determination of the bacterial contents is of little moment, whilst it is of great importance to ascertain whether bacteria are present which will develop in the malt extract or beer, and so possibly exercise a deleterious effect upon the liquor. Recognising this fact, Hansen[1] uses, instead of gelatine-peptone, sterilised wort and sterilised beer respectively as culture media.

By thus abandoning solid culture media for these liquid ones, Hansen has to adopt of course a totally different method of forming an approximate estimate of the number of microbes in the water under examination. The method he employs is in fact a modification of Miquel's (see p. 73), which we shall subsequently describe, but it will be convenient to indicate Hansen's precise mode of procedure with regard to brewing-waters at this stage.

Hansen takes 15 small flasks containing sterile malt-wort and 15 similar flasks with sterile beer, and to each of these he adds 1 drop (of known volume) of the water under examination, whilst to each of 10 further flasks of sterile wort and 10 of sterile beer respectively he adds $\frac{1}{4}$ c.c. of the same water. The 50 flasks to which the water has thus been added are kept at 25° C., and examined after 14 days to see how many of them have become turbid through growths. From the percentage number of turbid flasks an opinion can be formed as to the bacteriological fitness of the water for brewing, whilst an estimate of the number of micro-organisms capable of flourishing in these media can be arrived at in the following manner. Thus, taking the 10 flasks of

[1] 'Methode zur Analyse des Brauwassers in Rücksicht auf Mikro-organismen,' *Zeitschrift für das ges. Brauwesen*, 1888, No. 1. Also *Untersuchungen aus der Praxis der Gärungsindustrie*, München und Leipzig, 1892, p. 1.

wort to which $\frac{1}{4}$ c.c. of water respectively was added to represent in all 2·5 c.c. of water, and supposing only 4 of these 10 flasks became turbid, whilst the remaining 6 remained clear, the presumption would obviously be that each of the 4 turbid flasks had been rendered turbid by the multiplication of a single microbe introduced in the water added. If this supposition be granted, it follows that the 2·5 c.c. of water employed contained 4 microbes only capable of developing in the malt-wort or 1·6 microbe per 1 c.c. Should all the 10 flasks have become turbid, it would of course prevent any exact numerical deduction being made beyond the obvious one that each $\frac{1}{4}$ c.c. of water contained at least 1 developable microbe.

In this case, however, the 15 flasks, into each of which one drop of water had been introduced, would come into requisition, as in all probability only some of these would have become turbid, and in that case a numerical estimate of the developable micro-organisms in a given volume of water could be made on the same principles as indicated above. Of course, the exact volumes of water employed in this method of examination will have to be varied according to circumstances. A microscopic examination should also be made of the contents of those flasks which have become turbid, and if necessary further experiments conducted to ascertain whether the particular micro-organisms thus discovered may exert a deleterious effect on the wort or beer.

A large number of investigations have been made by Hansen and his pupils to compare the numbers of bacteria in brewing waters revealed by ordinary gelatine-peptone and wort-gelatine plates respectively, as well as by means of the wort and beer method described above.

The following table of results is taken from a recent paper by Holm [1] :—

Water No. 1

Plate cultures	Gelatine-peptone yielded about 8,000 colonies per c.c. (Mostly bacteria.)
	Gelatine to which wort had been added yielded about 14 colonies per c.c. (Moulds.)
Liquid cultures	Sterilised wort yielded about 5·4 colonies per c.c. (Bacteria and moulds.)
	Sterilised beer yielded about 0·8 colonies per c.c. (Moulds.)

Water No. 2

Plate cultures	Gelatine-peptone yielded about 350 colonies per c.c. (Bacteria.)
	Wort-gelatine yielded about 8 colonies per c.c. (Moulds.)
Liquid cultures	Sterilised wort yielded about 5·3 colonies per c.c. (Bacteria and moulds.)
	Sterilised beer yielded about 0·8 colonies per c.c. (Moulds.)

Water No. 3

Plate cultures	Gelatine-peptone yielded about 370 colonies per c.c. (Bacteria.)
	Wort-gelatine yielded about 4 colonies per c.c. (Bacteria.)
Liquid cultures	Sterilised wort yielded about 1·1 colonies per c.c. (Moulds and Torula (yeast).)
	Sterilised beer yielded about 0·4 colonies per c.c. (Moulds.)

Thus, in the case of the last water, the wort and beer revealed the presence of totally different organisms from those which made their appearance on the gelatine plate-cultures. It was pointed out by Hansen that the great practical utility of the substitution of sterilised wort and beer for gelatine media is that some organisms, which, from the brewer's point of view, it is

[1] 'Analyses biologiques et zymotechniques de l'eau destinée aux brasseries,' *Compte-rendu des travaux du laboratoire de Carlsberg*, vol. iii. livraison 2. Copenhagen, 1892. See also 'Sur les méthodes de culture pure et, spécialement, sur la culture sur plaques de M. Koch et la limite des erreurs de cette méthode,' *loc. cit.* vol. iii., livraison 1. 1891.

of primary importance to detect, do not develop at all in the gelatine media, and, if the latter only were employed, would therefore be entirely overlooked. Hansen states that he has frequently found that many saccharomycetes and other alcoholic ferments are in such a weakened condition in air, earth, and water that they are quite incapable of growing in gelatine, or, if they grow at all, develop very feebly, whilst when introduced into wort and beer they grow most luxuriantly.

In order to ascertain the value of a water for brewing purposes, it is of great importance to note whether the development of the organisms is slow or rapid. If the organisms present only commence to develop four or five days after inoculation, it may be taken that when exposed to the far more unfavourable conditions prevalent during brewing operations they would only develop with great difficulty, or possibly not at all. For in the experimental flasks they are present under very favourable conditions, no competing forms in the shape of the yeast cells disturbing their development, whilst the temperature, at any rate for the majority of the forms, is very suitable for their growth and multiplication.

Holm states as a result of a large number of investigations that, if, at the end of seven days, no growths make their appearance in the wort or beer, the examination may, for *practical* purposes, be closed, although it is quite possible that growths may yet develop subsequently. To still further simplify the investigation, it is proposed to employ sterilised wort only, as all organisms which develop in beer will also develop in the wort. In spite of the frequent presence of moulds, Holm states that they are only of secondary importance. The most dangerous organisms are the bacteria, and more especially those which are present

in the fermentation-cellar, although, as a rule, they are not able to develop in the beer during its sojourn in the store-cellar; but when the beer is drawn off, and thus aërated, and placed in bottles or small casks and exposed to a higher temperature, such bacteria may multiply with astonishing rapidity and cause great damage.

A slight modification of Hansen's method has been introduced by Wichmann[1] in order to give some numerical expression to the different vital energy possessed by the micro-organisms present in various waters. For this purpose he takes twenty-five small flasks, each containing 10 c.c. of sterile malt-wort, and twenty-five containing the same quantity of sterile beer; into twenty of each kind one drop (=·025 c.c.) of the water under examination is added,[2] whilst four further flasks of each kind receive respectively ·25, ·50, ·75, and 1·0 c.c. of the water, whilst the twenty-fifth tube of each kind is kept for control. The following arbitrary standard is then adopted to represent the relative fitness or unfitness of the water:—A water which renders the above four flasks of wort turbid in twenty-four hours, and the four flasks of beer within three days, is represented as possessing a degree of impurity 100. Lesser degrees of impurity are calculated from the length of time elapsing before each of the above four dilutions becomes turbid, by multiplying the number of the dilution by a constant factor, according to the time, and then adding these products together. Thus, in the case of the wort, if the turbidity appears on the first day the factor is ten, on the second day eight, on

[1] 'Biologische Untersuchung des Wassers für Brauereizwecke,' *Mittheilungen der Oesterr. Versuchsstation für Brauerei und Mälzerei.* Heft 5, 1892. (*Centralblatt für Bakteriologie*, vol. xiii., 1893, p. 207.)

[2] These twenty flasks, to which one drop of water has been added respectively, are intended to serve for Hansen's method as described on p. 68.

the third day six, on the fourth day four, and on the fifth day two. Taking a concrete example with wort, suppose No. 1 flask (10 c.c. wort + 1 c.c. water) becomes turbid on the second day, the product is (1 × 8); No. 2 flask (10 c.c. wort + ·75 c.c. water) becomes turbid on the third day, the product is (2 × 6); No. 3 flask (10 c.c. wort + ·5 c.c. water) also becomes turbid on the third day, the product is (3 × 6); No. 4 flask (10 c.c. wort + ·25 c.c. water) becomes turbid on the fourth day, the product is (4 × 4). Then adding these products together, we obtain

$$\begin{array}{cccccccc} (1 \times 8) & + & (2 \times 6) & + & (3 \times 6) & + & (4 \times 4) & \\ 8 & + & 12 & + & 18 & + & 16 & = 54 \end{array}$$

as the numerical expression for the energy with which the particular water is capable of producing growths in wort.

In the case of beer the above factors are multiplied by $\frac{5}{3}$, so that they are respectively

$$10 \times \tfrac{5}{3} = 17\,;\ 8 \times \tfrac{5}{3} = 13{\cdot}3\,;\ 6 \times \tfrac{5}{3} = 10\,;\ 4 \times \tfrac{5}{3} = 6{\cdot}7\,;$$
$$2 \times \tfrac{5}{3} = 3{\cdot}3\,;$$

and in the concrete example given above, if the results had been obtained with beer instead of wort, the numerical expression for the energy of growth possessed by the water in respect of beer would be

$$\begin{array}{cccccccc} (1 \times 13{\cdot}3) & + & (2 \times 10) & + & (3 \times 10) & + & (4 \times 6{\cdot}7) & \\ 13{\cdot}3 & + & 20 & + & 30 & + & 26{\cdot}8 & = 90{\cdot}1. \end{array}$$

Numerical Determination of Bacteria in Water (Miquel's Method)

In a previous chapter (see p. 28) an account is given of the method of isolating particular micro-organisms from any given material by means of the dilution method. In Miquel's process of water exami-

nation the same principle is utilised in the estimation of the number of bacteria in a given volume of water; it is carried out in the following manner:—About a hundred test-tubes, each containing 10 c.c. of sterile broth, are prepared; into the first tube, A_1, 1 c.c. of the water under examination is introduced by means of a pipette. Into a second broth tube, A_2, 1 c.c. is introduced from tube A_1; A_2 will then contain 11 c.c. of liquid, which is equally divided into a series of eleven broth tubes, marked respectively A_3, A^1_3, A^2_3, &c. The contents of A_3, containing, like A_2, 11 c.c. of liquid, is then equally divided amongst eleven more tubes marked A_4, A^1_4, &c. The number of such series of dilutions that must be prepared will depend upon the number of microbes supposed to be present in the water, the object being to ultimately obtain *a series of tubes, each of which shall not receive more than one microbe.*

All these broth-tubes, with the exception of A_2 and A_3 (the whole contents of which were divided amongst the two series of tubes A^1_3, A^2_3, ... and A^1_4, A^2_4 . . . .), are incubated for some days, or even weeks, at from 30° to 35° C. If all the tubes subsequently exhibit turbidity, it shows that the dilution has not been carried far enough, and the process must be repeated; or, if that is impossible (which, as regards water, must be the case, for the sample, after standing days or weeks, is rendered absolutely worthless through the multiplication of the water-bacteria in the interval), a fresh sample must be collected and the process repeated *de novo.*

If, on the other hand, in one of the several series of dilutions described above some tubes become turbid, whilst others remain clear, it is argued that the turbid ones have received only a single microbe apiece; and if this be granted, it is obvious that from the number of

turbid tubes the number of microbes in the volume of water represented by the particular dilution-series can be inferred. Thus the series of tubes A^1_3, A^2_3, A^3_3, ... A^{10}_3, has received in all ·1 c.c. of the original water, and, to take a simple case, if three out of these ten tubes became turbid, the other seven remaining clear, it would be inferred that ·1 c.c. of the water contained three microbes, or thirty microbes in 1·0 c.c.

It is obvious that this undoubtedly ingenious method possesses a number of grave disadvantages; thus it is not only exceedingly laborious, but the possibility of failure is very considerable, unless skill and judgment are employed in arranging the necessary degree of dilution required by the particular sample of water under examination. Moreover, it affords little or no immediate indication as to the particular varieties of microbes present in the water. On the other hand, some real advantages attach to this method, viz.: First, that the cultivation is made in a liquid medium in which some bacteria will thrive which would not develop on gelatine-plates; and second, that the incubation can be effected at any desired temperature, whilst with gelatine-plates the temperature of incubation cannot exceed about 22° C.; on this account it is in exceptional cases necessary to employ this dilution method for the examination of water.

In actual practice Miquel has more recently adopted what he calls the 'mixed process.' The water is diluted to, say, 100, 1,000, 10,000, or 100,000, &c. times its volume, according as the particular sample is supposed to contain a smaller or larger number of bacteria. After this has been done, from one to two drops are introduced into a flask with a large flat bottom (about 9 centimetres in diameter) containing a layer, about 2 millimetres in thickness, of sterile and liquefied

gelatine-peptone. The whole is then gently agitated, allowed to solidify, and incubated at 20–22° C., and the resulting colonies counted and examined in the usual way. Miquel uses about a dozen such flasks for each water examination. The number of bacteria originally present in the sample can then be calculated from the number of colonies which make their appearance in the gelatine-films of these flasks. Excepting in the use of the cumbrous and otherwise inconvenient conical flask in question, this so-called 'mixed process' of Miquel's does not differ in any single detail from the ordinary method of plate-culture as commonly practised, for it should be pointed out that preliminary dilution before plate-cultivation must invariably be resorted to in the case of all waters which, like sewage, polluted streams, &c., are very rich in bacterial life. Such preliminary dilution is best made with sterilised natural water, and not with sterilised distilled water, as the latter is liable to prejudicially affect some bacteria.

Numerical Determination of Bacteria in Water by Gelatine-cultures[1]

The method of pouring gelatine-plates has already been given (see p. 30), and it only remains here to describe the process as applied to water examinations. Gelatine-plates, small round covered dishes, or Esmarch-tubes, may all be employed for this purpose. The frequent presence of microbes in water which liquefy the gelatine renders the Esmarch-tubes less serviceable than

[1] For the *qualitative* determination of the bacteria present in any given water, besides examining the colonies on the gelatine-plates with a low power under the microscope (see p. 35) and inoculating particular colonies into gelatine-tubes &c., recourse must be had to the special methods described on pp. 267, 276, when typhoid or cholera bacteria are suspected of being present.

the gelatine-plates, whilst most convenient of all are the covered shallow dishes (commonly called Petri-dishes) already referred to (see p. 34). After the ice-plate has been arranged, and all the necessary preparations made for the pouring of plates, the sample of water is taken and violently shaken for several minutes, in order to disintegrate any aggregations of microbes, as well as to secure even distribution of the bacteria throughout the liquid. A definite quantity of the water is now removed by means of a sterilised and graduated pipette, and introduced into the gelatine-tube, which, during the operation, should be held in a slanting position. The cotton-wool stopper is then replaced and the contents gently agitated, and the stopper removed with all the precautions already described, and the plate poured in the usual manner. Supposing the water to be fairly pure as regards microbes, 1 c.c. may be taken for one plate, and ·5 c.c. for a duplicate. In all cases at least two plates must be poured of each sample of water. If the water is suspected of containing a large number of bacteria, then it will be necessary to dilute, say, 1 c.c. of it 50 or 100 or 500 times, as the case may be, before pouring the plates. For this purpose a small sterile stoppered bottle containing, say, 50 c.c. of sterilised natural water (not distilled water) may receive 1 c.c. of the original water. After thorough shaking, 1 c.c. from this bottle may be introduced into another similar bottle, and so on, until the attenuation is considered sufficient; plates may then be poured from the two last attenuations. As in all such manipulations success can only be attained after practice and much experience. The plates are incubated in the usual manner, and the counting of the colonies is conveniently carried out with the assistance of a counting apparatus (fig. 13).

This consists of a wooden stand A on which is sup-

ported at *a a a a* the transparent glass plate B. The middle portion C of this glass plate is etched out into squares, some of which, situated in various parts of the field, are further divided up into nine smaller squares. When the gelatine-plate is ready to be counted, B is raised, the gelatine-plate so placed that it is covered by the etched surface C, and a small magnifying glass resting on three

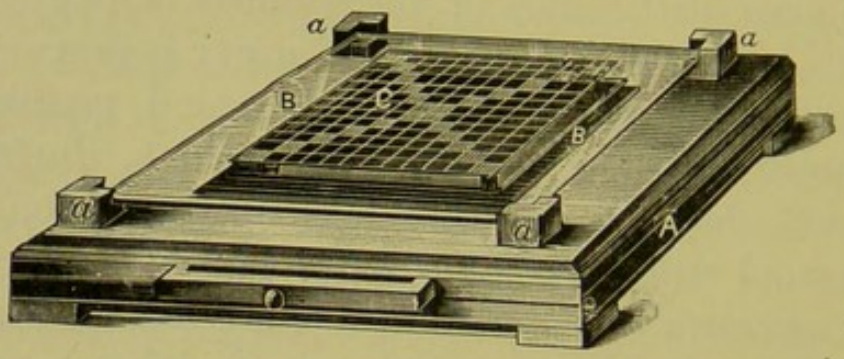


Fig. 13.—Wolffhügel's Counting Apparatus.

feet is placed on C, and the colonies enclosed by each square counted. If the colonies on the plate are too numerous to count individually, an approximate estimate may be made of their numbers by counting those contained in a few of the large squares (which is most accurately carried out by using the squares divided into the nine smaller squares) and then multiplying the average number on these squares by the total number of squares over which the gelatine-film extends; a very accurate result may be thus obtained if the water has been well mixed with the gelatine in the first instance. The number of colonies found is then calculated on 1 c.c. of the original water.

By using glass dishes [1] instead of plates there is much less risk of aërial contamination, whilst by introducing the water direct from the pipette into the melted gelatine in the dish the loss is avoided of those bacteria which must necessarily remain in the gelatine left ad-

[1] See note, p. 34.

hering to the tube when the mixture is made in the test-tube before pouring on to the plate or into the dish. Such loss is, however, quite insignificant, and does not materially influence the result. Another advantage obtained by substituting dishes for plates is in the greater ease with which the former can be transported. It is often advisable to undertake the bacteriological examination of a water on the spot, especially in cases where the investigation is required at a considerable distance from the laboratory, and whereas the transference of gelatine-plates would be almost impossible, the safe conveyance of such dish-cultures is attended with no difficulty whatever.

In connection with the use of pipettes for the measuring out of the water, care should be taken that they are sterilised in the same way as all pieces of glass apparatus in the hot-air oven. As it is very important that the pipettes should not be greasy, they must be thoroughly cleaned by soaking them successively in strong sulphuric acid, water, caustic soda, water, and hydrochloric acid, after which they are thoroughly washed and finally rinsed with distilled water. They are then placed, point downwards, in a cylindrical glass or tinned iron vessel covered with a beaker, and the whole sterilised in the hot-air oven. The beaker covering the pipettes is then just raised each time a pipette is removed for use, the pipette being of course held by its upper extremity, which will not come in contact with the water. Immediately after the pipette is finished with it should be placed in a beaker containing distilled water, or, better, strong sulphuric acid, especially if pathogenic organisms are believed to be present.

Detection of Anaërobic Micro-organisms in Water

In the various methods of water-examination described above, only those microbes, of course, which are capable of growing in the presence of air will be discovered, whilst for the investigation of anaërobic forms, which only flourish in the absence of free oxygen, the methods described on pp. 35, 38 must be employed. Particularly well adapted for such investigations is the method of placing an ordinary Esmarch-tube cultivation inside a larger closed tube containing potassium pyrogallate.

It must of course be borne in mind that there are many bacteria which grow both in the presence and in the absence of free oxygen, so that such microbes will give rise to colonies both in the aërobic and anaërobic cultures.

Photographic Record of Plate-cultures

A very simple and effective method of photographically reproducing the appearances of gelatine-plate cultures of bacteria may be mentioned before closing this chapter. It has been devised by De Giaxa,[1] and the accompanying figure gives some idea of the results obtained.

When the plate has been incubated for a sufficient length of time it is removed from the moist chamber, and its under-surface is carefully wiped with blotting-paper soaked in ether, in order to remove all traces of moisture. The plate is then placed on a piece of albuminised paper rendered sensitive by means of silver nitrate, just as is used in ordinary photographic operations. In order to

[1] 'Ueber eine einfache Methode zur Reproduction der Koch'schen Kulturplatten,' *Centralblatt für Bakteriologie*, vol. iii., 1888, p. 700.

obtain uniform contact between the sensitised paper and the plate it is advisable to interpose a piece of

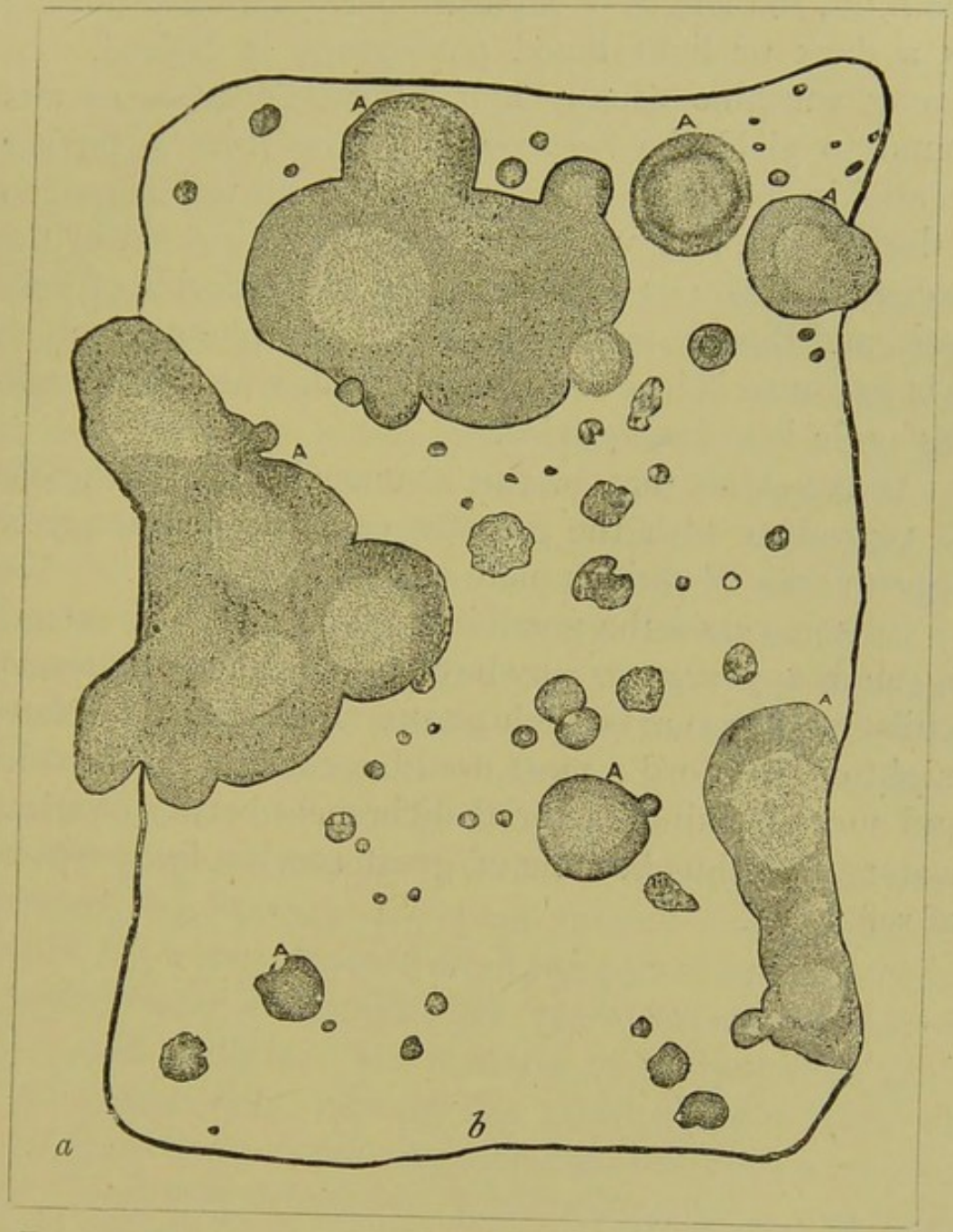


FIG. 14.—PHOTOGRAPHIC REPRODUCTION OF A GELATINE-PLATE CULTURE.
a, the glass plate; *b*, the gelatine film; A, colonies causing liquefaction of the gelatine.
(After de Giaxa.)

thick cloth between the paper and the board on which it rests. The plate is further covered with a thin glass bell-jar, in order to protect it from dust and other foreign particles.

The above manipulations are carried out in the dark room, and when all is ready the apparatus is placed in the light, the length of exposure being varied according as a dark or light toned photograph is desired. In strong sunshine usually half a minute's exposure was found to yield the best results. The print is further treated in the usual manner. The paper is removed to a dark room, and washed repeatedly to get rid of the excess of silver; it is then placed in a chloride of gold bath, and then in one of sodium thiosulphate, in which it is left until it has become well fixed, when after washing again it is finally dried.

Giaxa states that in this manner he has been able to reproduce with the greatest ease the characteristic appearances of various plate-cultures.

In some cases the possibility of permanently recording such appearances may be of much value and interest; whilst a collection of such photographic prints of plates might often afford a most useful record of the broader and more striking bacterial differences between various waters, and thus become of great service for purposes of reference.

CHAPTER IV

THE BACTERIAL CONTENTS OF VARIOUS WATERS

Before proceeding to apply the bacteriological methods to the investigation of the various processes of purification to which water is submitted on the small as well as on the large scale, thus endeavouring from the biological point of view to obtain some information as to the value of such different kinds of treatment, it will be necessary to gain some general idea of the numbers of micro-organisms which are to be found in waters derived from different sources. We may mention, however, that a large amount of additional information concerning the bacterial contents of various kinds of water will also be found in Chapter V.

As long ago as the year 1871 Burdon Sanderson[1] showed conclusively that both filtered and unfiltered water, ice-water obtained from the purest ice, and even distilled water which had not been recently prepared contained bacteria. The method of investigation pursued consisted in introducing these several kinds of water into flasks containing sterilised Pasteur's solution: if the solution subsequently became turbid it was concluded that bacteria were present; whilst if, on the other hand, they remained clear, the inference was of course made that no bacteria were contained in the liquids

[1] 'The Origin and Distribution of Microzymes in Water and the Circumstances which Determine their Existence in the Tissues and Liquids of the Living Body.' Thirteenth Report of the Medical Officer of the Privy Council, reprinted in the *Quarterly Journal of the Microscopical Society*, October 1871.

under investigation. Since these results were published a very large number of researches have been undertaken to obtain, by means of the modern methods of investigation, more precise information as to the bacterial contents of water, and the conditions under which micro-organisms not only gain access to, but exist in, various waters.

Snow.—Some interesting experiments on the bacterial contents of snow have been carried out in Russia by Janowski.[1] In the first place snow which had been lying for some time was examined; the superficial layers were removed and the sample taken from below, so as to ensure the absence of any disturbance through aërial contamination. The snow was allowed to melt in a test-tube, and plates were poured with the following results :—

1. On February 11, no snow having fallen on the previous day, 1 c.c. of snow-water yielded 3 organisms (mean of two experiments).

2. On February 15, no snow for 4 days, 10 per c.c. (mean of two experiments).

3. On February 24, no snow for 3 days, hard frost, 228 per c.c.

4. On March 2, no snow for 3 days, 178 per c.c. (mean of two experiments).

Thus, in spite of prolonged exposure to a low temperature, *e.g.*, after three days during which the thermometer only reached −16° C. in the middle of the day on February 24, the snow contained a considerable number of micro-organisms. Janowski also examined freshly fallen snow, and found varying numbers per c.c. In samples collected at a temperature of about −7·2° C. in the month of February he found from 34 to

[1] 'Ueber den Bakteriengehalt des Schnees,' *Centralblatt für Bakteriologie*, vol. iv., 1888, p. 547.

38 in 1 c.c.; on another occasion, when the temperature was about −11·1° C., 293 (mean of two experiments); again, about a week later, the temperature being about −12·2° C., 154 (mean of two experiments); and during a snow-storm, temperature −3·9° C., 301 were found in 1 c.c. (mean of two experiments). Schmelck [1] has examined the snow from a glacier in Norway at a height of 1,800 to 2,000 metres above the sea, and found 2 bacteria and 2 moulds per 1 c.c. But the same sample yielded, after standing for five or six hours in a warm room, from 70 to 80 per 1 c.c. This points of course to an extensive multiplication of the organisms taking place after the melting of the snow.

Ice.—An elaborate series of investigations on the bacterial contents of ice has been carried out in Berlin by C. Fraenkl.[2] Fraenkl examined the ice supplied by one of the ice companies in Berlin, and derived from the Rummelsburg Lake, situated above Berlin, and forming an expansion of the river Spree. This ice is usually collected when it has reached a thickness of from 15 to 20 cm., and is stored in a large cellar. Periodical examinations were made of this ice from the middle of February 1886 until the middle of April. It was found how exceedingly variable were the numbers present per c.c., ranging from 21 to as many as 8,800.

The multiplication of the bacteria which may take place after the melting of the ice and standing of the ice-water is also very striking. Thus a piece of ice was melted and immediately examined, and found to contain 1,020 organisms per 1 c.c.; this ice-water was allowed to stand for eleven days, and was then found to contain as many as 220,000 per 1 c.c.

[1] *Centralblatt für Bakteriologie*, vol. iv. p. 545.

[2] 'Ueber den Bacteriengehalt des Eises,' *Zeitschrift für Hygiene*, vol. i. p. 302, 1886.

Numerous other investigations of the ice supplied to Berlin are recorded, and in some instances as many as 25,000 micro-organisms per c.c. were discovered, whilst, on the contrary, in artificial ice, for the preparation of which distilled water was used, organisms were almost completely absent.

Still more recently Heyroth[1] has published a series of investigations on ice as obtained from various places in the neighbourhood of Berlin, and supplied by different companies for public consumption. The following table shows the places from which the ice was collected, as well as the number of bacteria contained in 1 c.c. of the ice-water.

Bacterial Contents of Ice (Heyroth)

Day of Investigation	Origin of the Ice	Number of bacteria in 1 c.c.
19. 9.85	Plötzen Lake	490
5.10.85	„	4,900
12.10.85	„	121
19. 9.85	Rummelsburger Lake and waters near Köpernick	425
5.10.85	„ „ „	210
12.10.85	„ „ „	1,150
12.10.85	Kaiser-bassin at the Navigation Canal, Spandau	634
12.10.85	Pond at Reinickendorf	2
15. 5.86	Plötzen Lake	1,835
17. 5.86	River Spree at Treptow	171
17. 5.86	„ „	30
17. 5.86	„ „	1,780
18. 5.86	Peaty pond-water from Lichtenberg Meadow	800
26. 5.86	„ „ „	500
15. 6.86	Flaken Lake at Erkner	448
15. 6.86	Peaty pond-water at Rummelsburg	392
15. 6.86	Pond-water at Tempelhof	1,510
15. 6.86	Peat-meadows at Rixdorf	2,040
15. 6.86	Lichtenberg Meadow and Lichtenberg Lake	2,750
29. 6.86	Lake Reinickendorf	47
29. 6.86	Weissen Lake	735
29. 6.86	Rummelsburg Lake and waters near Köpernick	765
29. 6.86	Pond-water (situation not specified)	14,400

[1] 'Ueber den Reinlichkeitszustand des natürlichen und künstlichen Eises,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. iv., 1888, p. 1.

These figures show again how very variable are the bacterial contents of ice, not only when derived from different sources, but also in those samples collected from one and the same place.

Heyroth states that to avoid all chance of the disturbance of his results by external contamination, he first broke up the ice, and then abstracted with sterile forceps a good-sized piece out of the middle of the block, which was thoroughly washed with hot sterilised distilled water, so as to melt the surface of the lump of ice, and ensure with still more certainty the absence of any accidental pollution. The piece to be examined was then slowly melted in a sterile test-tube, and 1 c.c. of the ice-water submitted to gelatine-plate cultivation.

Bordoni Uffreduzzi[1] has made an examination of the number of micro-organisms present in ice supplied to Turin. The water for this purpose is collected from the river Dora, and is frozen and supplied by various companies to the city. It was found that whereas the river water contained innumerable micro-organisms in the cubic centimetre, the ice derived from the same water contained on an average 580 microbes in the cubic centimetre. It should be mentioned that these ice companies abstract their water from two different places, some from the river before it enters the city, others from a point lower down and after it has received pollutions from the city. The above average result was obtained from the purer river water.

Although, according to Uffreduzzi, the ice always contained 90 per cent. less organisms than the river water, yet this reduction is not sufficient to render ice

[1] 'Die biologische Untersuchung des Eises,' *Centralblatt für Bakteriologie*, vol. ii. p. 489.

obtained from polluted sources a safe article for consumption. See also p. 252 in chapter on 'The Multiplication of Micro-organisms,' for experiments on the vitality of the typhoid bacillus and other organisms in artificially frozen water.

Hail.—The bacterial contents of hailstones have been examined by Bujwid,[1] and later by Foutin.[2] In both cases the hailstone was first carefully washed to rid it of chance external contamination and then melted and plates poured. Bujwid found in Warschau as many as 21,000 organisms per c.c., whilst Foutin found in St. Petersburg in 1 c.c. of hailstone water only 729. Bujwid mentions that the stone he examined was an unusually large one, being 6 cm. long and 3 cm. thick.

Rain.—Curiously but few determinations of the number of organisms in rain have been made. Miquel records having found 4·3 in rain water collected at Montsouris, therefore outside Paris, and 19 in a cubic centimetre in the middle of the city. Both experiments were made during a rainy season. The average number found in the rain water at Montsouris observatory for the three years 1883–86 was 4·3 bacteria and 4·0 moulds per c.c., which, with a rainfall of 60 c.c., signified, says Miquel, that about 5,000,000 micro-organisms fall annually per square metre surface in that locality.

If freshly fallen snow, snow from the regions of glaciers and ice, contain micro-organisms, it will be readily understood that waters which are exposed to contamination will contain very large numbers of bacteria.

[1] 'Die Bakterien in Hagel-Körnern,' *Centralblatt für Bakteriologie*, vol. iii. 1888, p. 1.

[2] 'Bakteriologische Untersuchungen von Hagel,' *ibid.* vol. vii. 1890, p. 372.

Thus Miquel[1] found as many as 40,000,000 per c.c. in the water which had been used for the soaking of clothes prior to the use of soap in the floating laundries of the Seine.

Blasius[2] found 2,980,000 in a water used for manufacturing purposes.

In an investigation of the drainage of Essen, Wahl[3] found from 1,686,000 to 5,248,000 in a c.c.

Raw sewage was found by one of us to contain as many as 26,000,000 per c.c.

During the last few years a very large number of experiments have been made to ascertain the bacterial contents of different rivers from which cities and towns derive their water supplies. It will be interesting in the first place to consider some of these examinations before entering into more detail concerning the treatment which such waters receive before distribution.

Rivers.—London, as is well known, derives the greater portion of its water-supply from the rivers Thames and Lea. These rivers were first made the subject of careful bacteriological observation by one of us,[4] and at the request of the Local Government Board these investigations were carried out systematically, and were published in the monthly reports furnished by the Board. The following table gives the number of micro-organisms found in the Thames and Lea, above the intakes of the several companies drawing from these sources, for each month during the years 1886, 1887, and 1888.

[1] 'De la Richesse en Bactéries des Eaux d'Essangease,' *Revue d'Hygiène*, vol. viii. p. 388.

[2] *Monatsheft für öffentliche Gesundheitspflege*, no. 5 and 6, 1885. Braunschweig.

[3] *Centralblatt für allgemeine Gesundheitspflege*, vol. i. Bonn, 1886.

[4] *Local Government Board Reports*, 1885, 1886, 1887, 1888; also '*Secret Friends and Foes*,' Percy Frankland, 1893.

River Thames Water collected at Hampton
Number of Micro-organisms obtained from 1 *c.c. of Water*
(Percy Frankland)

Month	1886	1887	1888
January . . .	45,000	30,800	92,000
February . . .	15,800	6,700	40,000
March . . .	11,415	30,900	66,000
April . . .	12,250	52,100	13,000
May	4,800	2,100	1,900
June	8,300	2,200	3,500
July	3,000	2,500	1,070
August . . .	6,100	7,200	3,000
September . .	8,400	16,700	1,740
October . . .	8,600	6,700	1,130
November . .	56,000	81,000	11,700
December . . .	63,000	19,000	10,600

River Lea Water collected at Chingford
Number of Micro-organisms obtained from 1 *c.c. of Water*
(Percy Frankland)

Month	1886	1887	1888
January . . .	39,300	37,700	31,000
February . . .	20,600	7,900	26,000
March . . .	9,025	24,000	63,000
April . . .	7,300	1,330	84,000
May	2,950	2,200	1,124
June	4,700	12,200	7,000
July	5,400	12,300	2,190
August . . .	4,300	5,300	2,000
September . .	3,700	9,200	1,670
October . . .	6,400	7,600	2,310
November . .	12,700	27,000	57,500
December . . .	121,000	11,000	4,400

From the above figures it will be seen that it is during the summer months that these waters are purest as regards micro-organisms, this being due to the fact that during dry weather these rivers are mainly composed of spring water, whilst at other seasons they receive the washings of much cultivated land.

In the following table Miquel[1] has stated for each

[1] *Manuel pratique d'Analyse bactériologique des Eaux*, p. 132, 1891.

month the average of the observations made by him during three years, 1887–90, of the water derived from the Seine at Ivry, the Marne at St.-Maur, and the Ourcq.

Bacterial Contents of the Seine, the Marne, and the Ourcq (Miquel)
Number of Micro-organisms in 1 *c.c. of Water*

Month	Seine at Ivry	Marne at St.-Maur	Ourcq
January . . .	52,670	75,960	143,370
February . . .	43,120	58,120	63,720
March . . .	34,710	57,750	47,780
April. . . .	38,640	16,310	22,660
May	12,930	12,890	29,340
June. . . .	28,150	14,270	7,340
July	14,130	10,450	7,730
August . . .	6,780	13,570	8,520
September . .	20,220	6,410	8,070
October . . .	22,350	11,860	12,560
November . .	37,720	95,590	135,700
December. . .	78,950	62,470	153,200
Average for the year	32,530	36,305	53,330

From this table it will be seen that the same phenomenon is observable as in the case of the Thames and the Lea, viz., that it is during the winter months that the largest, and during the summer that the smallest, number of bacteria are present in the water.

In another series of experiments Miquel has collected Seine water above and below Paris, and also at St.-Denis after it has received the drainage from Paris. He found that at Choisy, above the city, there were 300 in 1 c.c., at Bercy, in the immediate vicinity of Paris, 1,200, whilst at St.-Denis the numbers rose to 200,000 per c.c.

An examination of the water supplied to Lyons from the rivers Rhône and Saône has been made by G. Roux.[1] The river Rhône water is filtered through sand

[1] *Précis d'Analyse microbiologique des Eaux*, p. 258. Paris, 1892.

and gravel before delivery. The following are the results obtained :—

Filtered and Unfiltered River Rhône Water (G. Roux, 1890)

	Number of Micro-organisms per c.c.
Rhône (above Lyons)	75
Filter bed	7
Reservoir (low service)	18
Reservoir (high service)	26
Tap-water	60
Rhône (below Lyons)	800

The river Saône, on the other hand, is much richer in bacterial life, as will be seen from the following results, although less so than the rivers Seine, Marne, and Ourcq at Paris. The figures obtained are the mean of a large number of examinations made of this water.

River Saône above and below Lyons (G. Roux, 1890)

	Number of Micro-organisms per c.c.
Saône (above Lyons)	586
Saône (Mouton bridge)	1,594
Saône (Tilsitt bridge)	860
Saône (below Lyons)	4,280

The river Spree, which supplies Berlin, has been bacteriologically investigated by various workers, notably by Koch [1] in 1883, by Plagge and Proskauer [2] in 1885–86, and by Frank [3] in 1886–87.

Frank collected a large number of samples of the river in and below Berlin, to trace if possible the contamination which the Spree undergoes in its flow through the city.

The following table gives the result of these bacteriological observations. It should be mentioned in explanation of the places selected for the collection of the

[1] *Bericht der Deputation für die Verwaltung der Canalisationswerke*, Berlin, 1883.

[2] *Zeitschrift für Hygiene*, vol. ii. p. 401.

[3] *Ibid.* vol. iii. p. 355.

Table showing the number of Micro-organisms obtained from 1 *c.c. of Water taken from the River Spree in and below Berlin* (Frank)

Place of Collection		April 7, 1886	April 21, 1886	May 5, 1886	May 18, 1886	June 2, 1886	June 16, 1886	June 30, 1886	July 14, 1886	July 27, 1886	Aug. 11, 1886	Aug. 25, 1886
Within Berlin	Oberbaumbrücke	5,100	4,000	4,320	2,200	26,400	2,800	10,500	8,400	1,900	4,500	4,200
	Janowitzbrücke	6,200	7,200	3,000	11,800	24,900	6,300	8,200	18,800	13,200	19,000	9,300
	Friedrichsbrücke	11,700	7,500	5,800	5,800	7,000	5,700	8,800	11,500	130,000	27,000	15,400
	Ebertsbrücke	10,500	10,920	4,100	8,200	34,000	10,900	25,600	12,100	30,000	45,000	14,400
	Marschallbrücke	10,900	19,200	5,300	7,300	80,000	11,300	37,700	49,000	30,000	51,000	17,100
	Moltkebrücke	14,200	10,200	7,200	13,300	108,000	9,100	97,200	130,000	64,800	63,000	7,300
	Moabiterbrücke	24,700	9,500	5,400	11,200	63,000	16,500	36,400	98,000	72,000	90,000	52,300
Landwehr Canal	Hafenplatz	39,500	31,000	50,000	42,100	351,000	216,000	200,000	150,000	494,000	350,000	200,000
	Lichtensteinbrücke	46,100	92,700	32,100	63,700	1,392,000	165,000	38,700	121,000	320,000	90,000	42,500
Below Berlin	Ruhlebener Schleuse	—	45,600	97,400	255,000	1,250,000	136,800	140,000	610,000	182,000	200,000	90,000
	Spandau	—	—	38,200	370,000	50,000	123,500	230,000	848,400	130,000	190,000	70,000
	Pichelsdorf	—	52,500	40,500	250,000	893,800	192,000	7,200	470,000	65,000	110,000	23,500
	Gatow	243,000	64,000	54,900	104,000	117,600	486,000	290,000	52,800	400,000	144,000	200,000
	Cladow	24,600	30,200	2,900	62,800	—	270,000	290,000	1,044,000	330,000	180,000	473,000
	Sacrow	3,200	2,400	1,700	21,200	13,400	3,100	4,000	8,300	4,000	—	2,000

Place of Collection		Sept. 8, 1886	Sept. 22, 1886	Oct. 6, 1886	Oct. 20, 1886	Nov. 3, 1886	Nov. 17, 1886	Dec. 1, 1886	Dec. 15, 1886	Jan. 5, 1887	Feb. 2, 1887	March 2, 1887
Within Berlin	Oberbaumbrücke	7,000	6,700	1,900	2,600	10,300	8,100	5,900	4,800	2,000	8,300	65,000
	Janowitzbrücke	21,800	15,000	5,300	6,700	11,100	7,800	4,600	13,600	9,800	7,500	63,000
	Friedrichsbrücke	65,000	40,000	75,000	25,900	22,800	15,300	21,000	12,000	9,200	3,900	61,600
	Ebertsbrücke	144,000	154,000	50,000	22,800	132,500	8,400	10,100	19,000	14,300	3,200	61,600
	Marschallbrücke	136,800	108,000	16,600	15,000	36,500	6,100	4,800	15,800	6,500	3,500	27,300
	Moltkebrücke	385,000	143,000	53,200	18,600	240,000	5,800	5,500	32,600	8,600	4,500	104,000
	Moabiterbrücke	96,000	154,000	78,500	45,000	51,000	41,400	6,200	42,700	12,800	6,800	105,000
Landwehr Canal	Hafenplatz	200,000	260,000	165,000	300,000	216,000	198,000	27,000	43,100	63,000	23,300	143,000
	Lichtensteinbrücke	540,000	356,400	162,000	224,600	—	35,100	19,600	41,100	38,800	24,400	100,800
Below Berlin	Ruhlebener Schleuse	200,000	550,800	132,000	457,600	252,000	252,000	37,000	48,500	32,600	19,900	126,000
	Spandau	400,000	2,520,000	767,000	—	48,800	167,200	39,300	50,200	33,700	21,800	170,100
	Pichelsdorf	120,000	435,000	204,000	300,000	525,000	90,200	6,300	18,000	30,700	19,600	119,700
	Gatow	200,000	246,400	24,000	9,000	13,800	9,000	29,000	15,500	12,200	18,200	176,000
	Cladow	650,000	112,000	9,500	8,400	24,700	6,300	23,900	8,900	10,600	219[1]	96,000
	Sacrow	24,700	6,800	12,400	11,100	20,300	3,900	4,500	8,000	6,600	2,200	29,600

[1] Ice water.

various samples that Oberbaumbrücke, Janowitzbrücke, Friedrichsbrücke, Ebertsbrücke, Marschallbrücke, Moltkebrücke, Moabiterbrücke, are all within Berlin and in the main-flow of the Spree. Hafenplatz and Lichtensteinbrücke are on the middle and lower end of the Landwehr canal, which is largely polluted with sewage; whilst below Berlin are the Ruhlebener Schleuse, situated $1\frac{1}{4}$ mile from the centre of the town; Spandau, half a mile further down and below the junction of the rivers Spree and Havel; Pichelsdorf, at the head of the Havel Lake; Gatow and Cladow, farther on; whilst Sacrow lies at the foot of the lake. As regards the bacterial contents of the Spree above Berlin we must refer to Proskauer's experiments, the spot selected being the intake of the waterworks.

River Spree, abstracted at the Intake of the Berlin Waterworks
(Proskauer)

Date	Number of Micro-organisms contained in 1 c.c. of water	Date	Number of Micro-organisms contained in 1 c.c. of water
1886		1886	
April 6	17,000	October 15	8,950
„ 13	8,400	November 1	4,060
„ 20	11,100	„ 15	8,400
„ 27	1,500	December 1	4,060
May 4	750	„ 15	3,460
„ 17	1,860	1887	
June 1	4,000	January 3	1,690[1]
„ 15	3,670	„ 15	7,200[1]
July 1	7,000	February 1	5,840[1]
„ 15	6,000	„ 15	760
August 2	11,000	March 1	2,860
„ 16	3,360	„ 15	2,740
September 1	4,000		
„ 15	16,000	Maximum	17,000
October 1	4,500	Minimum	750

[1] Ice.

These experiments of Proskauer's show that already at the waterworks' intake the Spree contains a large number of micro-organisms, and that in its flow through Berlin these numbers, according to Frank, constantly

increase in consequence of the numerous sources of pollution to which it is exposed all along its course. After Pichelsdorf, however, there is a diminution in the number of bacteria apparent, until at Sacrow the bacterial contents bear comparison with those found in the river at its entrance into Berlin.

Pichelsdorf is, as we have said, situated at the commencement of the Havel Lake, which is 7 miles in length, and between 0·28 and 1·2 mile wide, whilst Sacrow lies at the other end. The cause of the greater bacterial purity of the water here is due, on the one hand, to the influx of a considerable volume of spring water, which flows into the lake and serves to largely dilute the polluted river-water, whilst, on the other hand, the diminished rate at which the water moves, after passing from the narrow river-bed to the lake-expansion, admits of the subsidence of some of the matters in suspension; and a certain proportion of the microbes consequently sink to the bottom and disappear. This sedimentation of bacteria in water is of the utmost importance, and will be referred to at greater length later on, when the purification of water is being discussed.

Prausnitz[1] has examined the bacterial condition of the river Isar before and after it receives the drainage of Munich, and the results are given in the following table:—

Bacterial Composition of the River Isar above and below Munich (Prausnitz)

Description	Number of Colonies obtained from 1 c.c. of water
Above Munich	531
Near the entrance of the principal sewer	227,369
Ismaning (13 kilometres from Munich)	9,111
Erching (22 kilometres from Munich)	4,796
Freising (33 kilometres from Munich)	2,378

[1] *Der Einfluss der Münchener Canalisation auf die Isar.* München, 1889. See also p. 373.

The time occupied by the Isar in flowing from Munich to Freising is about eight hours, and in this time, therefore, by far the greater proportion of the organisms introduced into the river at the point of the sewage outfall disappear.

Since the experiments on the Spree by Frank, and those on the Isar by Prausnitz, an exhaustive investigation has been made by Schlatter[1] of the variations in the bacterial condition of the river Limmat before and after it receives the drainage of Zürich. As in the above experiments, various spots along the river's flow were selected, and a series of samples collected and submitted to plate-cultivation. The observations were made during the months of January to April 1889, and the following were the places chosen for the sampling of the water:—

(1) Stadtmühle. This is pure Limmat water, and is just above the influx of the sewage.

(2) Left current of drainage water after admixture with the river,
(3) Middle current of drainage water after admixture with the river,
(4) Right current of drainage water after admixture with the river,
} about 40 metres below the entrance of the drain.

(5) Water coming from the Hardmühle about 450 metres lower down than Nos. 2, 3, and 4.

(6) Hard Fähre about 300 metres lower down than No. 5. There is a silk factory between this and No. 7, but experiments showed that the bacterial disturbance caused by it was insignificant.

(7) Höngger Brücke. 2½ kilometres below Nos. 2, 3, and 4.

(8) Engstringer Brücke. 4 kilometres below No. 7.

(9) Kloster Fährli. 600 metres below the bridge (No. 8).

(10) Dietikon Fähre. 10½ kilometres below Nos. 2, 3, and 4.

The whole distance covered by the investigations, from the Lake of Zürich to Dietikon, is 14 kilometres.

[1] 'Der Einfluss des Abwassers der Stadt Zürich auf den Bacteriengehalt der Limmat,' by Carl Schlatter. *Zeitschrift für Hygiene*, vol. ix. p. 56, 1890.

We have gathered together the results in the following table :—

Table showing Bacterial Composition of the River Limmat before and after it receives the Drainage of Zürich (Schlatter)

Place of Collection	Number of Micro-organisms contained in 1 c.c. of water							
	Jan. 21	Jan. 23[1]	Jan. 28[2]	Jan. 30[3]	Feb. 4[4]	Feb. 6[5]	Feb. 13[6]	Feb. 15[7]
(1) Stadtmühle	1,620	1,530	1,020	1,910	1,860	1,290	200	In consequence of the ice no samples could be taken
(2) Wipkinger Brücke Left current	1,580	78,460	310,650	1,990	49,740	153,470	36,070	
(3) Wipkinger Brücke Middle „	27,040	43,300	570,280	448,940	88,450	788,700	28,800	
(4) Wipkinger Brücke Right „	17,500	1,220	820	311,060	43,140	24,500	141,800	
(5) Hard-Mühle	25,600	22,300	—	31,460	29,860	25,270	9,650	
(6) Hard-Fähre	18,370	9,700	18,000	12,870	24,260	15,060	—	
(7) Höngger Brücke	—	16,160	—	—	24,450	18,270	3,830	
(8) Engstringer Brücke	—	—	—	—	—	15,050	1,430	24,320
(9) Kloster Fährli	—	—	—	—	—	2,220	900	17,260
(10) Dietikon	—	—	—	—	—	—	800	14,180

Place of Collection	Number of Micro-organisms contained in 1 c.c. of water							
	Feb. 18[8]	Feb. 20[9]	Feb. 25[10]	Feb. 27	Mar. 4	Mar. 6	Ap. 26[12]	Apr. 30
(1) Stadtmühle	3,350	6,870	1,250	160	1,020	1,220	1,050	—
(2) Wipkinger Brücke Left current	57,700	35,470	20,570	2,810	43,980	200,900	9,430	7,400
(3) Wipkinger Brücke Middle „	37,650	62,620	24,230	17,370	136,080	236,640	10,540	8,470
(4) Wipkinger Brücke Left „	18,580	231,200	16,010	35,950	217,700	326,550	17,610	17,900
(5) Hard-Mühle	5,480	53,470	1,290	340	18,650	28,700	2,220	3,000
(6) Hard-Fähre	2,300	51,140	1,540	440	9,840	16,160	2,640	4,380
(7) Höngger Brücke	2,350	6,370	860	150	7,250	14,270	4,000	1,650
(8) Engstringer Brücke	400	2,950	800	250	7,500	5,700	2,540	5,560
(9) Kloster Fährli	—	2,470	1,980	—	3,600	1,940	5,100	3,950
(10) Dietikon	—	1,080	510	250	3,900	3,130[11]	5,100	3,550

[1] Rain had fallen on the previous day.
[2] Snow had fallen in the morning. The samples 2, 3, 4, were collected from on board a boat in the afternoon.
[3] Thaw had set in. [4] Snow had fallen on the previous day.
[5] Snowstorm in the morning previous to taking of sample. [6] Slight fall of snow on previous day.
[7] Thaw and slight fall of snow. [8] Thaw. [9] Thaw. Snow over-night, rain the day before.
[10] Snow over-night and in the morning.
[11] River-navigation works were being carried on above Dietikon. [12] Rain.

The average number of micro-organisms in the water from the Lake of Zürich is from 100 to 200 in the c.c. The river Limmat flows out of the lake, and the samples collected below Zürich and after it has passed through the city, but before it receives its drainage, show that already in its flow through Zürich it has become more or less contaminated. The increase in the number of bacteria is, however, very much more marked below the point of the sewage outfall, and after

rain or snow has fallen the numbers show a decided increase. This is especially exhibited in the experiments of January 28 and 30 and February 6 and 15, the latter experiment being particularly instructive in this respect, as many as 24,000 micro-organisms being found at Engstringen in 1 c.c. of water. At Dietikon, after a flow of 10 kilometres, the bacterial condition of the water approaches very often to that of the river before the influx of sewage.

On April 26 and April 30 the flow of the river was three times as rapid as usual, indicating a larger volume of water passing along the river-bed. Thus the effect of the sewage discharge was not bacterially so marked, whilst the sedimentation of the micro-organisms in consequence of the more rapid flow of the water was not so complete, the number at Engstringen, in proportion to those found at Wipkinger, being larger than usual.

Thus, again, is shown the diminution in the number of bacteria which takes place during the flow of a river, the bacterial purification of the water through sedimentation taking place in this instance without the assistance of a lake-expansion, as in the case of the river Spree at the Havel, but during the uninterrupted flow of the river along its course.

According to Moers,[1] the Rhine at Mühlheim contained in 1885 the following numbers of micro-organisms in a c.c. :—

Bacterial Condition of the River Rhine at Mühlheim (Moers)

April 12 . .	17,300	August 28 .	23,000
June 6 . .	21,000	October 15 .	20,500
July 12 . .	21,000	November 1 .	21,300

The river Main has been examined by Rosenberg[2]

[1] 'Die Brunnen der Stadt Mühlheim a. Rh. vom bakteriologischen Standpunkte aus betrachtet,' *Ergänzungsheft z. Centralblatt für allgem. Gesundheitspflege.* Bd. 2. Heft 2.

[2] *Archiv für Hygiene*, p. 448, 1886.

above and below Würzburg, with the following results:—

Bacterial Composition of the River Main above and below Würzburg (Rosenberg)

Number of Micro-organisms contained in 1 c.c. of water

February		*March*	
Above	Below	Above	Below
520	15,500	740	17,000
355	2,950	830	13,500
680	16,000	2,050	9,500
780	6,600	610	7,100
640	6,400	910	23,000
720	18,000	640	35,000
565	17,200	950	11,500
1,020	14,000	800	18,500
680	22,000	525	17,000
March		385	16,200
		750	15,000
680	15,000	830	19,000

The river Neva, within St. Petersburg, was found by Poehl[1] to contain in September, 1883, 1,500; in October, 312; later in October, 1,524; in November, 6,500; later in November, 3,146. The Little Neva contained, when examined in September, 4,836; in October, 5,772.

Tils[2] has made an exhaustive investigation of the Freiburg water supply, the main part of which is derived from a mountain-stream, which is conveyed to a reservoir, from whence pipes distribute it to the town. It was found that bacterially the water was purer when examined from the reservoir than when taken at the source, showing that here, again, the sedimentation of bacteria takes place. Taking the average of a large number of experiments, the supply derived from the reservoir was found to contain twelve organisms in the cubic centimetre, whilst the same water, after passing

[1] *Mittheilungen aus dem physiol. chemischen Laboratorium zu St. Petersburg*, part I., 1884.

[2] *Zeitschrift für Hygiene*, vol. ix. p. 282.

through the service pipes, contained on an average forty-four.

This increase in the number of micro-organisms in service-pipes will be referred to later on in connection with experiments made by one of us, on the deep-well water supplied to London by the Kent Company.

The microbial condition of the rivers Ure and Ouse above York has been investigated by one of us,[1] with the following results :—

Bacterial Composition of the Rivers Ure and Ouse above York
(Percy Frankland)

Description	Number of Colonies obtained from 1 c.c. of water
Taken from the river Ure, above Ripon, twenty-seven miles above intake of the York waterworks. The river Ure rises on the western extremity of the North Riding of Yorkshire, passing on its way only small towns and villages.	1,800
Collected about sixteen miles above intake of the York waterworks, and about three miles below Boro'bridge, and ten miles below Ripon, both discharging some sewage into the Ure.	33,400
Collected from the river Ouse opposite the intake of the York waterworks. Between the points of collection of No. 2 and this sample there is no town and only one small river, the Nidd.	31,200

The river Dee, from which Aberdeen obtains its water-supply, has been quite recently (July, 1892) bacteriologically examined by one of us,[2] with the following results :—

Above Braemar the Dee was found to yield only eighty-eight micro-organisms in 1 c.c., and is, therefore, bacteriologically of great purity. The next sample was taken from the river below Old Mar Castle, and after thorough incorporation of the tributary Cluny (which

[1] 'Recent Bacteriological Research in connection with Water Supply,' *Jour. Soc. Chem. Ind.*, 1887, Percy Frankland.

[2] *Report to the Corporation of Aberdeen*, Percy Frankland, 1892.

receives the sewage of Braemar). This accession is marked by a large increase in the number of microbes, there being found as many as 2,829 in a c.c. Another sample was taken above the Bridge of Ballater, and it shows that the number of micro-organisms has been reduced by rather more than one-half in the flow from Old Mar Castle to this point, for only 1,139 were found in the c.c. A sample examined from the Dee 100 yards below the Ballater sewage-pond outfall, on the other hand, shows a large increase in the number of micro-organisms, as many as 3,780 being obtained from a c.c. The cause of this increase, notwithstanding the very small quantity of liquid coming from the pond at the time (10 o'clock in the morning), is not far to seek; for as many as 235,000, 273,000, and 26,000,000 organisms were found in the effluent from the sewage-pond, and the sewage-outfall channel above the pond at Ballater respectively. On following the Dee down, a sample was taken above the junction of the Neil Burn, Kincardine o' Neil, and it was found that the micro-organisms had again fallen to about the same number as above Ballater, the number amounting to 938 in the c.c. Below the entrance of the Neil Burn, which at the time of the investigation was a mere dribble, the number of micro-organisms had again perceptibly risen, 1,860 being found in the c.c. The Neil Burn itself, twelve yards below sewage-outfall, was found to be very rich in micro-organisms, as many as 1,200,000 being obtained in the c.c. The river on reaching Invercannie, however, again exhibits just about the same number of micro-organisms as above Kincardine o' Neil or above Ballater, only 950 being found in the c.c. The total number of miles of the river's flow which was covered by the examination was upwards of 40.

The river Dee thus affords a most perfect example of the repeated pollution and repeated restoration of a

stream to a state of comparative bacterial purity. This case of the Dee is, moreover, specially interesting, as the amount of polluting material gaining access at these several points is so small in comparison with the volume of the stream itself that, as a matter of fact, it was found impossible by means of chemical analysis to detect any material alteration in the composition of the river, even immediately below each of the above sources of contamination. By means of the bacteriological examination, however, as already indicated, each source of pollution was found to have produced an unmistakable, although transitory, mark on the water of the stream. This repeated restoration of the Dee to a state of comparative bacterial purity is undoubtedly, partially at any rate, attributable to dilution through the increase in volume which takes place during its course. Indeed, in nature it is practically impossible to exclude this complicating factor in the phenomenon of the self-purification of rivers.

Having obtained some idea of the bacterial contents of river-water, we will now turn our attention to the examinations which have been made of well-waters, upon which many public and private water-supplies are dependent.

Well-water.—A great many wells have been investigated for micro-organisms, but the data in most cases are so incomplete that no reliance can be placed upon the numbers found being representative of the well in question. The principal source of error in this respect is due to the samples in most cases having been collected irrespective of whether the pumps had been in operation or not. Now, the sides of a well are covered with a slimy deposit, and if the water has remained stationary for any length of time ample opportunity is afforded for the extensive multiplication of micro-organisms taking place on such surfaces. It is

obvious, therefore, that on pumping being resumed these surfaces will be washed down, and consequently in the first pumping the water will receive a great influx of micro-organisms, and it will be only after pumping has been going on continuously for some time that *a sample representing the bacterial condition of the water gaining access to the well is obtainable.* The following example will show the power of multiplication possessed by the organisms in deep-well water.[1]

Bacterial Contents of Well-water before and after standing
(Percy Frankland)

Description	Number of Micro-organisms obtained from 1 c.c. of water: Date of Collection, April 14, 1886	April 15, 1886, after standing for 1 Day at 20° C.	April 17, 1886, standing 3 Days at 20° C.
Kent well sunk into chalk .	7	21	495,000

Heraeus[2] examined a well-water which had only been very little used during the previous thirty-six hours, and found 5,000 organisms per c.c.; but after the well was emptied by continuous pumping a second sample was collected, after an interval of half-an-hour, and only 35 in the c.c. were present. Similar results were also obtained by C. Fränkel.[3]

Maschek[4] has also shown the effect of pumping on the microbial contents of well-water thus:—

Effect of Pumping on the Bacterial Contents of Well-water
(Maschek)

	Number of Micro-organisms obtained from 1 c.c. of water
Well-water after continuous pumping for fifteen minutes .	458
„ „ „ „ „ many hours .	140
„ later	68
„ after continuous pumping for fifteen minutes .	578
„ „ „ „ „ many hours .	179
„ later	73

[1] 'On the Multiplication of Micro-organisms,' Percy Frankland, *Proc. Roy. Soc.* 1886.

[2] 'Ueber das Verhalten der Bacterien im Brunnenwasser,' *Zeitschrift für Hygiene*, vol. i. 1886.

[3] 'Ueber Brunnendesinfection u. Keimgehalt des Grundwassers,' *ibid.* vol. vi. p. 32, 1889.

[4] *Kubel-Tiemann-Gärtner* '*Wasser*,' pp. 576–77. Braunschweig, 1889.

Rubner[1] has conducted a series of investigations on the bacterial contents of a well in Marburg, in which some instructive information was obtained as to the manner in which the microbial condition of such water may be altered by the introduction of any disturbing causes.

The well selected for these experiments was situated in an unused cellar of the Hygienic Institute at Marburg. The soil in which the well was sunk was loam, mixed with a small quantity of sand, and only a small yield of water was obtained. The well was never used, and to all intents and purposes therefore was stagnant water. Although stagnant, this water contained a remarkably small number of bacteria, as is seen by reference to the following table :—

Bacterial Contents of a Stagnant Well-water (Rubner)

Date	Number of Micro-organisms per c.c.	Temperature
June 9, 1886	1,000	9·0° C.
July 3 „	1,220	9·2° C.
„ 10 „	850	10·1° C.
„ 12 „	863	10·1° C.
„ 17 „	980	10·4° C.
„ 24 „	1,220	10·4° C.
„ 27 „	1,171	10·8° C.
August 25, 1886	1,620	11·3° C.
September 21, 1886	960	11·8° C.
January 11, 1887	1,142	8·1° C.

In the course of his experiments, wishing to ascertain the effect on the bacterial composition of the water of disturbing the mud at the bottom of the well, Rubner first took a sample of the water, and then stirred up the mud until the water became turbid. The following table records the results of his observations :—

[1] 'Beitrag zur Lehre von den Wasserbacterien,' *Archiv für Hygiene*, vol. xi. p. 365, 1890.

Bacterial Composition of Well-water before and after Disturbance
(Rubner)

Date	Number of Micro-organisms per 1 c.c.	Appearance
Before stirring up the sediment, August 25, 1886	1,620	—
At 1 o'clock, August 25	1,475,000	Very turbid
„ 4 „ „	196,000	„
„ 6 „ „	180,000	„
Middle of the Day, August 27 . . .	44,100	Normal
„ „ September 21 . . .	960	„

Rubner, in pointing out the enormous increase in the number of bacteria after disturbance of the sediment, states that the well had only been made from a half to three-quarters of a year previous, and had been so carefully constructed that no external pollution could possibly gain access to it. The disturbance thus created was evident in the bacterial examination for some weeks later, and it was only on September 21 that the number of micro-organisms again became normal.

It is important to notice in connection with these experiments that the bacteria found belonged to the well itself, and were not introduced by the accession of polluting materials. The presence of the large number of bacteria denotes, therefore, in such a case, disturbance, not contamination of the well, whilst, conversely, a small number of bacteria denotes prolonged quiescence of the water, and not necessarily absence of contamination.

It is therefore imperative that in the examination of well-water for micro-organisms due attention should be paid to the conditions recently prevailing, *e.g.* as to whether the well has been recently pumped or disturbed, or has been unused for a longer or shorter period; and unless these circumstances are duly taken into consideration, the most misleading and erroneous

conclusions as to the bacterial condition of the actual source of supply will be arrived at (see p. 62).

The following table gives the results of examinations of the deep-well water obtained from the chalk and supplied to London by the Kent Company during the years 1886, 1887 and 1888[1]:—

Bacterial Contents of Deep-well Water obtained from the Chalk and supplied to London by the Kent Company (Percy Frankland)

Wells	Number of Micro-organisms obtained from 1 c.c.											
	1886											
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Bath Well .	—	—	—	—	—	—	—	—	—	—	12	10
Garden Well	—	—	—	—	—	—	—	—	—	—	—	—
New Well .	—	5*	44*	7*	8*	4*	12*	9*	5*	3*	—	11
	—	—	—	—	—	—	—	—	—	160*		
Supply . .	43	149	38	47	101	39	48	13	25	283	196	66
										405		
	[In those cases marked with an asterisk the name of the particular well examined was not recorded.]											
	1887											
Bath Well .	9	19	80	26	27	12	14	5	5	7	3	6
Garden Well	48	20	4	4	—	24	18	—	8	—	5	12
New Well .	12	10	5	12	20	14	8	59	27	30	65	67
Supply . .	82	75	140	163	50	26	44	116	115	357	40	68
	1888											
Bath Well .	6	47	6	33	7	17	8	—	8	4	34	—
Garden Well	5	19	8	4	27	71	5	—	10	9	18	—
New Well .	12	4	5	7	8	20	4	3	—	96	19	—
Supply . .	55	81	15	69	139	219	32	42	52	55	54	63

N.B.—The samples referred to as 'supply' were obtained from the mains in the company's district, and represent not only mixtures of the water from the several wells mentioned in the table, but also with the water of other similar wells belonging to the company. This water has also passed through the service reservoir, and, as would be anticipated, therefore the numbers found are often considerably in excess of those discovered in the samples taken directly from the wells. In nearly all those cases in which larger numbers, such as 160, 59, 65, 67, &c., were discovered in the well-waters, it was found on inquiry that there had been some irregularity in the work at the well, such as recent repairs, interrupted pumping, &c.

An artesian well at Mainz was found by Egger to contain four organisms in a c.c. Hueppe found also only four in a deep well attached to a slaughter-house in Wiesbaden, whilst in an investigation of some artesian

[1] *Reports to the Local Government Board on the Monthly Bacteriological Examinations of the London Water Supply*, 1886, 1887, 1888, Percy Frankland; also *Proc. Roy. Soc.* 1893, vol. liii. p. 178.

wells in Kiel, Breunig[1] found numbers varying between 6 and 30 in the c.c. Kowalsky found in some deep-wells in Vienna an average of from 18 to 33.

All these investigations show what a high degree of bacterial purity is possessed by these deep-wells, and when the enormous depths of porous strata are taken into consideration through which the water gaining access to such wells has to pass, this poverty in microbial life is not to be wondered at. In a subsequent chapter on the purification processes, both artificial and natural, to which water is submitted, this subject will be dealt with in greater detail.

Spring-water.—The number of micro-organisms in spring-water, protected from chance contamination, is usually very small, whilst in some cases no organisms at all have been discoverable. Thus Libbertz[2] found no organisms in several samples of spring-water supplying Frankfort-on-Main, whilst in other samples collected after heavy rain he only obtained from 50 to 60 in 1 c.c.

Freimuth[3] examined on four different occasions the spring-water supplied to Dantzig, and only once found micro-organisms, and then but two in a c.c.

Buchner[4] also found no organisms in a spring from Giesing, whilst at another time 5 were obtained. In the Brunnthal spring the same author found from 4 to 35 in a c.c.

In spring-water near Jena, Fürbringer found from 32 to 156 in a c.c.

A spring in the Upper Greensand near Reigate was found by one of us to contain 8.[5]

[1] *Bakteriologische Untersuchung des Trinkwassers der Stadt Kiel im August und September*, 1887. Kiel, 1888.

[2] *Arbeiten aus dem Kaiserlichen Gesundheitsamt*, vol. i. p. 560, 1886.

[3] *Ibid.* p. 558.

[4] *Loc. cit.* p. 551.

[5] 'New Aspects of Filtration and other Methods of Water Treatment. Percy Frankland, *Jour. Society of Chemical Industry*, 1885.

Maschek investigated three springs in Leitmeritz, and found as many as from 700 to 3,000 organisms in 1 c.c. These springs are supplied by a mountain stream, but, according to Maschek, the drainage from dung-heaps gained access to them. Outside the town the examination of four springs gave an average of from 2 to 27 in 1 c.c.

In springs at Zürich, Cramer[1] found from 9 to 3,425 organisms in 1 c.c.

As in all other respects, so also in their bacterial character, spring and deep-well waters are thus practically identical.

Mineral Springs.—Fazio[2] examined some of the mineral springs which abound in the vicinity of Castellamare (Bay of Naples), and found very few micro-organisms.

The samples investigated were taken from the chalybeate springs of Castellamare, the carbonated sulphur springs of Telese (containing carbonic acid gas and sulphuretted hydrogen), and the alkaline springs of Acetosella and Muraglione respectively. All these waters are rich in carbonic acid gas, and Fazio attributes the small numbers found at the source, and the larger number at a distance from the source, respectively to the concentrated condition of this gas in the water where it rises and the subsequent partial dissipation and consequent diminution in the amount present during the passage of the water. But impurities in the shape of soil and aërial microbes must gain access to the water during its flow, which would also account for the increase in the number found at a distance from the source.

[1] *Die Wasserversorgung von Zürich*, 1885.
[2] *I Microbi delle Acque Minerali*, Napoli, 1888.

Mineral Springs (Fazio)

Description of Water		Number of Micro-organisms found in 1 c.c.
Chalybeate springs of Castellamare	Acqua rossa, about 60 metres from the source. Temp. 14° C. . . . . March 1888	12
	„ „ „ „ May 1888 .	28
	Acqua del Mulino, also at some distance from the source. Temp. 15° C. . . . March 1888	42
	„ „ „ „ May 1888 .	18
Carbonated sulphur springs	Collected at the source. Temp. 20–22° C. (Telese.) June 1887 .	1
	„ „ „ (Castellamare.) May 1888 .	4
Alkaline springs	Collected at the source. Temp. 12–14° C. (Acetosella.) May 1888 .	15
	„ „ „ under more favourable external conditions . . . . Sept. 1888 .	2
	Springs at Muraglione. Temp. 16–18° C. (The exact spot is not mentioned.) . May 1888 .	19
	„ „ „ „ June 1888 .	21
	„ „ „ „ Sept. 1888 .	45

None of the micro-organisms found were pathogenic to animals. For the bacterial condition of artificial mineral waters, see pp. 235–242.

Lakes.—In examining the water direct from any particular lake, due attention must be paid to the fact that the numbers of micro-organisms vary according as the sample is taken near the shore or in the middle of the lake. In fact, the condition of the water may vary greatly in different parts. Fol and Dunant [1] have shown that the water of the Lake of Geneva contains as many as 150,000 in a c.c. taken near the shore, whereas in a c.c. examined from the middle of the lake only 38 were found.

Some recent observations by Karlinski [2] confirm these experiments. He studied the distribution of micro-organisms in the Borke Lake near Konjca in

[1] *Recherches sur le Nombre des Germes vivants que renferment quelques eaux de Genève.* Genève, 1884.

[2] 'Zur Kenntniss der Vertheilung der Wasserbakterien in grossen Wasserbecken,' *Centralblatt für Bakteriologie*, 1892, vol. xii. p. 220.

Herzegovina. This lake is situated about 403 metres above the Adriatic Sea, and is partially surrounded by high mountains, from which it is fed with snow-water. The average of a number of examinations showed that when the water was abstracted from the surface of the lake about 200 m. distant from the shore, 4,000 micro-organisms were found in 1 c.c.; whilst close to the shore, in the immediate neighbourhood of water-reeds, which abound all along the banks, as many as 16,000 in a c.c. were present. Karlinski also examined the water at different depths, and here great differences were observable. These examinations were conducted anaërobically as well, so that the maximum number of microbes was detected. Whilst on the surface 4,000 were present, at a depth of 5 m. hardly 1,000 were found in the c.c. At a depth of 10 m. there were rarely more than 600, and still lower down, 12 to 16 m., there were only from 200–300 present in the c.c. At the bottom of the lake, when the mud was stirred up, there were as many as 6,000 in the c.c. The above figures were obtained from an average of sixty observations.

The Lake of Zürich, according to Cramer,[1] was found to contain during the months of October and December in the year 1884, and January, 1885 (taking the average of fifty examinations), 168 in a c.c. In June, 1884, an average of forty-two investigations yielded only 71 in a c.c.

The Lake of Lucerne was found to contain from 8 to 51 in a c.c. The latter number was obtained from a shallower part of the lake, where the water was also disturbed by steam-boat traffic.

Loch Katrine water, as delivered in Glasgow, was

[1] *Die Wasserversorgung von Zürich.*

found[1] on July 6, 1892, by one of us to contain 74 in 1 c.c.

The Loch of Lintrathen,[2] which lies at the foot of the Grampians, and supplies the greater portion of Dundee, when examined from the service pipe in this city, was found to yield the following numbers :—

Bacterial Contents of Loch of Lintrathen Water supplying Dundee
(Percy Frankland)

Date of Collection	Number of Micro-organisms obtained from 1 c.c. of water
June 22, 1892	110
„ 23 „	149
„ 30 „	290
July 2 „	94
„ 4 „	114
„ 11 „	279
„ 17 „	177
„ 21 „	77
„ 29 „	155
Oct. 18 „	260

Thus the Loch of Lintrathen, taking the average of the examinations made, contains 170 micro-organisms in a c.c.

More recently we have examined the water just as it issued from the Loch itself, and found (June 10, 1893) 30 bacteria per c.c. On the other hand, a sample taken on the same day near the shore, and just where a large quantity of algæ were suspended in the water, exhibited as many as 4,000 per c.c.

The Tegeler Lake,[3] on the other hand, contained an average of from 127 to 890 in a c.c. when examined from June 1885 to April 1886.

Breunig found in the Schulensee, which is an expansion of the river Eider, 874, 772, and 760 micro-organisms in a c.c.

[1] Percy Frankland. *Proc. Roy. Society*, 1893, vol. liii. p. 225.

[2] *Ibid.*

[3] 'Bericht über die Untersuchung des Berliner Leitungswassers. Plagge und Proskauer, *Zeitschrift für Hygiene*, vol. ii. p. 401.

Thus, as far as the bacterial contents of lakes has been investigated, their waters appear to contain a remarkably small number of micro-organisms, which is what we should anticipate also, considering the opportunities which are offered for prolonged subsidence. Lakes should thus generally contain fewer micro-organisms than the streams which supply them, and this expectation was recently verified in a striking manner by one of us in the case of the Loch of Lintrathen, referred to above, and the two principal burns which enter it, thus :—

Inzion Burn, just above where it enters Lintrathen, June 10, 1893, contained				1,700	bacteria per c.c.
Melgam Burn	„	„	„	780	„
Water issuing from Lintrathen	„	„		30	„

The phenomenon is the more significant from the fact that chemically the waters of the burns were distinctly superior to that of the loch, the greater part of the loch water having doubtless been derived from these streams when they were both chemically and bacterially much inferior to what they were at the time of the collection of the above samples, which was during a prolonged and almost unprecedented drought, a condition most favourable to the purity of such streams.

Sea-water.—The bacterial contents of sea-water is a subject of obvious interest, but one on which but very little information was until recently available. Even now, practically, the whole extent of our knowledge is based upon the results obtained by two investigators, De Giaxa[1] and Russell,[2] both of whom conducted their experiments in the Gulf of Naples.

De Giaxa, whose investigations only incidentally included some determinations of the number of bacteria

[1] *Zeitschrift für Hygiene*, vol. vi. 1889, p. 186.

[2] *Ibid.* vol. xi. 1891, p. 177.

in sea-water, found as many as 298,000 in a c.c., but the sample was taken only 50 metres from the entry of the Chiatamone Canal (an excessively foul water containing the sewage of a large part of Naples); at a distance of 350 metres the number fell to 26,000, whilst at 3 kilom. only 10 were discoverable.

Russell,[1] whose researches in this particular direction were much more extended, examined samples taken from depths of 75 to 800 metres, at distances of 4 to 15 kilom. from the shore, and found from 6 to 78 microbes in 1 c.c. taken from the surface, and from 3 to 260 at various depths below.

The following table, whilst showing the particular distances from land as well as the various depths at which the samples were taken, brings out very clearly that, whilst the total number of bacteria in sea-water is comparatively small, there appears to be no marked decrease in the numbers corresponding to the greater distance from land of the water selected for examination, all the samples, be it observed, having been collected at a distance of upwards of 4 kilometres from the shore, and thus beyond the reach of any littoral influences.

The Distribution of Bacteria in Sea-water both Vertically and Horizontally (Russell)

Depth of the sea-bottom at places of collection	Distance from land	Number of bacteria in 1 c.c. of water taken from the surface	Number of bacteria found in 1 c.c. of water taken at various depths							
m.	km.		75 m.	100 m.	150 m.	200 m.	250 m.	300 m.	500 m.	800 m.
75	4	64	57	—	—	—	—	—	—	—
100	6	22	3	5	—	—	—	—	—	—
150	9	8	—	—	10	—	—	—	—	—
200	11	26	—	260	—	112	—	—	—	—
250	10	15	—	—	—	—	10	—	—	—
300	11	78	—	20	—	—	—	5	—	—
500	15	6	—	—	—	—	53	—	23	—
800	6	30	—	—	—	—	—	—	—	3

[1] *Zeitschrift für Hygiene*, vol. xi. 1891, p. 177.

Thus the nearest point to the land at which a sample was taken was 4 kilom., where the sea had a depth of 75 metres, and hence the chance of any disturbance in the normal bacterial contents of this sea-water from accidental contamination from the shore is very slight; and even if any such source of error should arise, it would at most be only likely to take place during or after violent storms blowing from the coast.

Examination of sea-mud revealed the presence of very large quantities of bacteria. It was found, however, that the numbers steadily diminished up to a certain point with the greater depth at which the samples were abstracted, this being especially noticeable in the immediate vicinity of the coast. After 250 m. and up to 1,100 m. no further important reduction was observed.

Number of Bacteria found at various depths in 1 *c.c. of sea-mud and* 1 *c.c. of sea-water respectively* (Russell)

Depth in metres	Water	Mud
50	121	245,000
85	57	285,000
100	10	200,000
140	10	70,000
200	59	70,800
250	31	27,000
300	5	24,000
400	30	22,000
500	22	12,500
825	31	20,000 [1]
1,100	—	24,000 [1]

[1] Russell states that these higher figures must not necessarily be regarded as indicating a larger number of bacteria as being present at the greater depth, but are to be attributed rather to slight *local* variations, as the samples were collected over as wide an area as possible.

As regards the varieties of organisms present, it was ascertained that more than half appeared only to belong to this mud, and were not discoverable in the sea-water itself; three individual microbes in particular were especially characteristic of this mud, as much as 35 per cent. of the total number of colonies found on

gelatine-plates consisting of these three forms (see pp. 454–456).

Russell has more recently [1] extended his investigations to an examination of the sea-water and mud in the vicinity of Wood's Holl, Massachusetts. The number of microbes present in these more northern and cooler waters was markedly less at this point than in the Mediterranean. The slime from Buzzard's Bay yielded an average of 10,000 to 30,000 bacteria in 1 c.c., which Russell says represent but a small fraction of those present in the Mediterranean mud at equal depths. Here again two species were found to be specially prevalent in the water, together with two or three other forms occasionally met with. The mud also contained these two prevailing water forms, but another form, an indigenous slime bacillus, occurred in such large numbers as to make up from thirty to fifty per cent. of the whole quantity present.

Samples of mud were also obtained, about 100 miles from the shore at the depth of 100 fathoms, on the edge of the great continental platform skirted by the Gulf Stream. These samples are the farthest from land that have ever been bacteriologically examined, and bacteria were present in large numbers; moreover, the two prevailing species present were identical with those obtained near the shore at Wood's Holl.

Russell mentions that the *Cladothrix intricata* (see p. 517) was only rarely met with, whereas in the Mediterranean mud it was frequently found.

On comparing these results with those already referred to for fresh-water lakes, it will be seen that the distribution of bacteria in the two cases is substantially similar, both the lake and the ocean at a

[1] *Botanical Gazette*, vol. xvii. p. 312. 1892.

distance from land being characterised by poverty in bacterial life.

Having now obtained some idea of the bacterial contents of various waters, we must direct our attention to a consideration of some of the numerous methods which may be adopted for removing them.

CHAPTER V

THE PURIFICATION OF WATER FOR DRINKING PURPOSES

Sand Filtration.—It is obvious that, with the information which the bacteriological investigation of water has furnished us, the subject of water-purification must be approached from an entirely novel point of view; that, whereas formerly the chemical standard was the only one which could be appealed to as a guarantee of the suitability or not of a water for domestic supply, we have now a far more delicate test as to the efficiency of the purification processes employed in the biological examination to which a water can be submitted. Thus, perhaps, a concrete example will most clearly illustrate how the purification of water must now be regarded. Supposing that a water, derived from a source which is altogether unimpeachable as regards contamination with animal matters, is yet so highly impregnated with vegetable constituents as to be unpalatable, the question will arise how this water may be treated so as to free it from this blemish and render it suitable for drinking purposes. In a case of this kind it is obvious that chemical purification will be of paramount importance, whilst the removal of organic life from the water will be of less pressing consequence. On the other hand, if water which is known to have received sewage matters (and the *entire* exclusion of such from supplies drawn from rivers is practically impossible) is to be supplied for dietetic use, and if this water, as is so often

the case, is not objectionable on account of the absolute quantity of organic matter, as revealed by chemical analysis, which it contains, but only because of the suspicious origin of a part of this organic matter, then it is evident that in the purification of such water the point to be taken primarily into consideration is how the organic life it contains can be reduced to a minimum.

In estimating the value of such processes of purification, it has hitherto been customary to assume that those processes which effect the greatest chemical improvement in water may also safely be considered to be biologically the most excellent; and, conversely, that those processes which effect little or no reduction in the proportion of organic impurity are not calculated to be of any service in removing organized matters.

The following chemical analyses of river-water before and after sand-filtration will sufficiently explain how, for example, the process of sand-filtration found comparatively little favour as long as the chemical analysis was the principal basis on which a judgment as to the hygienic value of water-filtration could be formed.

Chemical Analysis of Water of River Ouse before and after Sand-filtration (Percy Frankland)

Results of Analysis expressed in parts per 100,000

—	River Water	Ditto after Sand-filtration
Total solid matters	28·40	26·20
Organic carbon	·123	·119
„ nitrogen	·025	·022
Ammonia	0	0
Nitrogen as nitrates and nitrites	·077	·089
Total combined nitrogen	·102	·111
Chlorine	1·6	1·6
Hardness (Temporary)	11·5	10.9
Hardness (Permanent)	7·1	7·1
Hardness (Total)	18·6	18·0

Such slight chemical improvement as is here shown

indicating that sand-filtration had comparatively little effect on the dissolved matters in water, led to *à priori* speculation that it would be unlikely to remove bacteria and other minute forms of life. During the early growth of the belief in the communication of zymotic diseases by micro-organisms the process of filtration in general was viewed with great distrust, for it was not unnaturally assumed that the extremely small dimensions of these living creatures would admit of their passing through the comparatively large interstices of ordinary filtering media, with the same sort of facility that vehicles can thread their way along a crowded thoroughfare.

It is less than ten years ago since this latter point—the bacteriological side of the question—was for the first time put to the test of accurate experimental investigation, and in the interval a large amount of work has been done both in this country and on the Continent, which has placed the subject of water-purification on an entirely new basis.

London Water Supply: Rivers Thames and Lea.—The process of sand-filtration was first practised in connection with the London water supply in the year 1839, and although it was admitted that by its means the water was rendered clear and bright to the eye, yet, as already pointed out, beyond that there was no public confidence in its efficiency as a purifying agent. In the year 1885 Koch's gelatine-process of water examination was first introduced into this country by one of us[1] and applied to the London water supply, and the following table[2] gives the results of these first investigations :—

[1] 'The Removal of Micro-organisms from Water,' Percy Frankland. *Proc. Roy. Soc.* p. 379. 1885.

[2] 'Water-purification, its Biological and Chemical Basis,' Percy Frankland. *Transactions of Institution of Civil Engineers*, 1886.

Bacterial Contents of various Waters as supplied to London, 1885 (Percy Frankland)

Colonies obtained from 1 *c.c. of water*

—	Jan.	Feb.	Mar.	May	June		Sept.	Oct.	Nov.	Dec.
River Thames										
Hampton (unfiltered) .	—	—	—	—	—	—	1,644	714	1,866	4,650
Chelsea	8	23	10	14	22	81	13	34	33	9
West Middlesex . .	2	16	7	3	—	26	2	2	5	15
Southwark . . .	13	26	246	24	—	47	18	24	32	73
Grand Junction . .	382	57	28	3	21	18	43	40	40	134
Lambeth . . .	10	5	69	30	—	38	103	26	26	124
River Lea										
Chingford Mill (unfiltered) . . .	—	—	—	—	—	—	—	—	954	2,831
New River . . .	7	7	95	3	—	27	3	2	11	18
East London . .	25	39	17	21	—	22	29	53	14	317
Deep Wells										
Kent (well at Deptford)	—	—	—	—	6	—	—	6	8	5
,, Supply . .	10	41	9	20	26	—	14	18	—	7

The astonishing effect of sand-filtration, as practised by the London water companies, is here shown for the first time, and we find the first record of the actual reduction in the number of micro-organisms effected in the following table :—

1885	Thames	Lea
September	97·8 per cent.	—
October	96·5 ,,	—
November	98·9 ,,	98·5 per cent.
December	98·5 ,,	88.8 ,,

At the request of the Local Government Board regular bacteriological examinations were made by one of us[1] of the water supplied to London by the various water companies, and the results of these investigations for the years 1886, 1887 and 1888 are recorded in the following tables, together with the percentage reduction in the number of micro-organisms effected :—

[1] *Local Government Board Reports.* Eyre and Spottiswoode.

London Water Supply (Percy Frankland)

RIVERS THAMES AND LEA

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation, 1886

Name of Supply	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average for year
THAMES													
Thames water, unfiltered (Hampton)	45,000	15,800	11,415	12,250	4,800	8,300	3,000	6,100	8,400	8,600	56,000	63,000	—
Chelsea . . .	159	305	299	94	59	60	59	303	87	34	65	222	—
West Middlesex .	180	80	175	47	19	145	45	25	27	22	47	2,000	—
Southwark . .	2,270	284	1,562	77	29	94	380	60	49	61	321	1,100	—
Grand Junction .	4,894	208	379	115	51	17	14	12	17	77	80	1,700	—
Lambeth . . .	2,587	265	287	209	136	129	155	1,415	59	45	108	305	—
Reduction per cent.	95·6	98·6	95·3	99·1	98·8	98·9	95·6	94·0	99·4	99·4	99·8	98·3	97·6
LEA													
Lea water, unfiltered (Chingford) . .	39,300	20,600	9,025	7,300	2,950	4,700	5,400	4,300	3,700	6,400	12,700	121,000	—
New River . .	363	74	95	60	22	53	46	55	17	10	32	400	—
East London . .	224	252	533	269	143	445	134	243	165	97	248	280	—
Reduction per cent.[1]	99·4	98·8	94·1	96·3	95·2	90·5	97·5	94·3	95.5	98·5	98·0	99·8	96·5

[1] These reductions apply only to the East London supply.

London Water Supply (Percy Frankland)

Rivers Thames and Lea

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation, 1887

Name of Supply	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov	Dec.	Average for year
Thames													
Thames water, unfiltered (Hampton)	30,800	6,700	30,900	52,100	2,100	2,200	2,500	7,200	16,700	6,700	81,000	19,000	—
Chelsea . . .	5,300	81	171	55	49	190	106	44	73	64	187	86	—
West Middlesex .	258	27	96	110	32	123	40	87	82	28	53	113	—
Southwark . .	4,900	428	1,325	360	61	196	119	70	84	130	152	133	—
Grand Junction .	7,500	612	443	109	48	103	35	78	15	80	55	80	—
Lambeth . . .	1,200	188	884	103	53	521	108	733	85	96	1,120	198	—
Reduction per cent.	87·6	96·0	98·1	99·7	97·7	89·7	96·7	97·2	99·6	98·8	99·6	99·4	96·7
Lea													
Lea water, unfiltered (Chingford) . .	37,700	7,900	24,000	1,330	2,200	12,200	12,300	5,300	9,200	7,600	27,000	11,000	—
New River . .	508	72	133	38	16	31	33	15	23	25	41	39	—
East London . .	6,700	100	182	127	105	1,200	194	104	169	148	190	456	—
Reduction per cent.[1]	82·2	98·7	99·2	90·5	95·2	90·2	98·4	98·0	98·2	98·1	99·3	95·9	95·3

[1] These reductions apply only to the East London supply.

London Water Supply (Percy Frankland)

RIVERS THAMES AND LEA

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation, 1888

Name of Supply	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average for year
THAMES													
Thames water, unfiltered (Hampton)	92,000	40,000	66,000	13,000	1,900	3,500	1,070	3,000	1,740	1,130	11,700	10,600	—
Chelsea . . .	127	152	54	38	43	63	37	32	36	14	82	71	—
West Middlesex .	60	146	408	158	71	56	27	11	26	33	31	16	—
Southwark . .	177	766	742	47	47	24	35	27	106	35	167	136	—
Grand Junction .	90	349	617	56	77	40	15	4	20	16	25	208	—
Lambeth . . .	189	820	321	157	64	140	55	33	92	27	126	151	—
Reduction per cent.	99·9	98·9	99·4	99·3	96·8	98·1	96·8	99·3	96·8	97·8	99·3	98·9	98·4
LEA													
Lea water, unfiltered (Chingford) . .	31,000	26,000	63,000	84,000	1,124	7,000	2,190	2,000	1,670	2,310	57,500	4,400	—
New River . .	27	90	169	77	37	60	11	13	—	15	70	91	—
East London . .	2,038	780	359	193	209	266	253	57	64	63	49	141	—
Reduction per cent.[1]	93·4	97·0	99·4	99·8	81·4	96·2	88·4	97·2	96·2	97·3	99·9	96·8	95·3

[1] These reductions apply only to the East London supply.

Thus, on the average, out of every 100 micro-organisms present in the untreated river-water, there were removed by the water companies before distribution in the case of the

	1886	1887	1888	
Thames . . .	97·6	96·7	98·4	micro-organisms
Lea (E. London Co.)	96·5	95·3	95·3	,,

With regard to the removal of this large percentage proportion of the micro-organisms through the treatment adopted by the water companies, we may appropriately quote the following words from a paper read by one of us at the York Congress of the Sanitary Institute in 1886 [1]:—

'Although the organisms thus removed are probably in general perfectly harmless, it must not be supposed that their removal is of no importance, for it must be remembered that the micro-organisms which are known to produce disease, and which are termed pathogenic, do not in any way differ from the ordinary organisms in water so as to render it probable that they would behave differently in the process of filtration; but, on the contrary, there cannot be any serious doubt that their behaviour under these circumstances would be precisely similar. Now such disease-organisms frequently do gain access to water, and it is obviously of the greatest importance to ascertain what sort of impediment this process of filtration, which is so largely practised, offers to their passing on to the consumer.'

'By means of this bacteriological examination it is thus possible to obtain a far more satisfactory knowledge of the kind of filtration which water has undergone than by a mere appeal to the eye of the observer,

[1] 'Filtration of Water for Town-supply,' Percy Frankland. *Transactions of the Sanitary Institute of Great Britain*, vol. viii. 1886.

and the vague terms "turbid," "slightly turbid," "clear," and the like, which have hitherto been employed to describe whether the filtration of water has been satisfactory or not, must now be replaced by this scientific and important standard which I have described.'

These investigations further brought to light some very interesting points in connection with sand-filtration, and have indeed placed that process on a sound basis by exhibiting what are the principal factors in determining its efficiency. Thus, amongst the London water companies there are seven employing sand-filtration, and in the works of each the process has undergone to a great extent an independent evolution, so that in no two of them is the process at the present time carried on under precisely similar conditions. This, for experimental purposes, peculiarly fortunate circumstance enabled one of us to institute a comparison between the results achieved in the several modifications of the general process. In this connection we may quote from a paper read by one of us before the Institution of Civil Engineers in the year 1886, the conclusions which we then arrived at having been fully verified by further observations made both in this country and abroad since that date.

An examination of the tables of bacteriological results shows 'that there is a certain uniformity in the position which the various companies occupy as regards freedom from micro-organisms, and on referring to the statistics of the various companies published in Sir Francis Bolton's "Manual of the London Water Supply," it is found that there is an unmistakable relationship between this position of each company and certain factors in the mode of working, which might be anticipated from theoretical considerations.

'The factors which, in my opinion,[1] are more especially calculated to influence the number of micro-organisms present in the distributed water are the following:—

'1. Storage capacity for unfiltered water.
'2. Thickness of fine sand through which filtration is carried on.
'3. Rate of filtration.
'4. Renewal of filter-beds.

'1. *Influence of storage capacity for unfiltered water.*—The influence which this factor may exercise upon the organised matter in water is manifold. In the first place, through greater storage capacity, the necessity of drawing the worst water from the river is avoided, a matter which in the case of a river like the Thames, which is liable to frequent floods, is of great importance. During the period of storage subsidence takes place, the water becoming poorer in suspended particles of all kinds. Again, in these storage reservoirs a process of starvation may go on, for the organisms present in the impounded water find themselves imprisoned with a limited amount of sustenance, which they rapidly exhaust and then perish in large numbers, falling to the bottom. This phenomenon is sufficiently familiar to all who have made the cultivation of micro-organisms a subject of study.

'2. *Influence of thickness of fine sand.*—That the thickness of the filtering stratum should exercise an important influence on the number of micro-organisms passing through the filter will be sufficiently obvious to everyone. In referring to my laboratory experi-

[1] 'Water-purification, its Biological and Chemical Basis,' Percy Frankland. *Transactions of Institution of Civil Engineers*, 1886.

ments on filtration, I have already pointed out that comparatively thin strata of various materials are capable of largely, and sometimes wholly, removing the micro-organisms in the water passing through them, but that this power is gradually lost; it is only reasonable to suppose that a thicker stratum will lose this power less rapidly than a thinner one. In estimating the thickness of the filtering stratum, the fine sand only should be taken into consideration, as it is only this portion of the filter which can have any effect in the removal of micro-organisms.

'3. *Influence of rate of filtration.*—That the removal of micro-organisms is less perfect when the rate of filtration is increased, and *vice versâ*, has been illustrated by the results obtained in my laboratory experiments, already referred to.

'4. *Influence of renewal of filter-beds.*—As already pointed out, even the most perfect filtering media sooner or later lose their power of retaining micro-organisms, and hence the importance of frequent renewal is sufficiently apparent.

'In considering how the differences in these various factors, which the statistics of the water companies exhibit, may be expected to influence the results obtained in the removal of the micro-organisms, attention must be restricted to the five companies drawing water from the Thames, as it is only these which have approximately the same raw material to deal with; for from the tables it is seen that the amount of organic life found in the River Lea at the intake of the East London Company is often very different from that in the Thames at Hampton, and the difference in the case of the New River Company is doubtless even still greater, besides the problem being there complicated by the admixture of a very considerable proportion of deep-well water.

The close proximity of the intakes of the five Thames companies, however, furnishes a favourable opportunity for instituting a comparison.

'The factors in the mode of working, which have been pointed out as of special importance in exercising an influence on the result obtained are given in the following table, the figures being taken from the statistical table given in Sir F. Bolton's "London Water Supply'" 1884:—

More important Factors in mode of Treatment by Thames Water Companies

Name of Company	Average daily supply in millions of gallons	Storage capacity in millions of gallons	Average storage in days (Calculated)	Rate of filtration per square foot in gallons per hour	Thickness of fine sand	Renewal of filter-beds (Calculated). Proportion of total acreage cleaned per month
Chelsea . .	9·5	140·0	14·7	1·75	4 ft. 6 in.	0·59
W. Middlesex .	12·8	117·5	9·2	1·5	3 ft. 3 in.	0·90
Southwark .	19·9	66·0	3·3	1·5	3 ft. 0 in.	0·90
Grand Junction	14·1	64·5	4·6	1·75	2 ft. 6 in.	0·81
Lambeth . .	14·2	128·0	9·0	2·0	3 ft. 0 in.	0·50

'By means of this table the five companies may now be classified with respect to each of the four factors in question, thus:—

Company	Storage capacity	Thickness of fine sand	Rate of filtration	Renewal of filter-beds
Chelsea	1	1	3	4
West Middlesex . .	2	2	1	1
Southwark . . .	5	3	1	1
Grand Junction . .	4	5	3	3
Lambeth . . .	3	3	5	5

'From this, the general order of merit, as regards these factors, can be deduced, by taking the average position of each company, thus:—

Company	Average position	Order of merit
Chelsea	2·25	2
West Middlesex	1·5	1
Southwark	2·5	3
Grand Junction	3·75	4
Lambeth	4·0	5

'From the theoretical considerations here instituted, it would be anticipated, therefore, that, dealing with the same raw material, the West Middlesex Company should, on the whole, obtain the best average result, from a biological point of view, and that the results obtained by the other four companies would follow in the order of Chelsea, Southwark, Grand Junction, and Lambeth.'

In putting the above statement, which was made in the year 1886, to the test of the experience gained during the whole of the three years 1886, 1887, and 1888, over which our systematic investigations extended, it is found that the several companies stood as follows:—

Average number of Micro-organisms found in 1 *c.c. of Thames Supplies delivered in London*

Company	1886	1887	1888	Mean of 3 years
Chelsea	146	534	62	247
West Middlesex	234	87	88	136
Southwark	524	663	192	460
Grand Junction	630	763	126	506
Lambeth	475	441	181	366

Thus the average results taken over the three years are almost in complete harmony with theoretical anticipations, the only discrepancy (which admits of a ready explanation) being that the Lambeth Company is out of place with regard to the Southwark and Grand Junction Companies.

We may, perhaps, compare the results achieved by the several companies more satisfactorily by arranging

them each month in order of merit (*i.e.* as regards freedom from micro-organisms), and then taking the average position of each during the three years, thus :—

Micro-organisms

—	Chelsea	West Middlesex	Southwark	Grand Junction	Lambeth
1886					
January	1	2	3	5	4
February	5	1	4	2	3
March	3	1	5	4	2
April	3	1	2	4	5
May	4	1	2	3	5
June	2	5	3	1	4
July	3	2	5	1	4
August	4	2	3	1	5
September	5	2	3	1	4
October	2	1	4	5	3
November	2	1	5	3	4
December	1	5	3	4	2
Mean	2·9	2·0	3·5	2·8	3·8
1887					
January	4	1	3	5	2
February	2	1	4	5	3
March	2	1	5	3	4
April	1	2	5	4	3
May	3	1	5	2	4
June	3	2	4	1	5
July	3	2	5	1	4
August	1	4	2	3	5
September	2	3	4	1	5
October	2	1	5	3	4
November	4	1	3	2	5
December	2	3	4	1	5
Mean	2·4	1·8	4·1	2·6	4·1
1888					
January	3	1	4	2	5
February	2	1	4	3	5
March	1	3	5	4	2
April	1	5	2	3	4
May	1	4	2	5	3
June	4	3	1	2	5
July	4	2	3	1	5
August	4	2	3	1	5
September	3	2	5	1	4
October	1	4	5	2	3
November	3	2	5	1	4
December	2	1	4	5	3
Mean	2·4	2·5	3·6	2·5	4·0
Mean of 3 years	2·6	2·1	3·7	2·6	4·0

From the above it appears then that the average position during the three years of the

Chelsea	was	2·6
West Middlesex	„	2·1
Southwark	„	3·7
Grand Junction	„	2·6
Lambeth	„	4·0

thus again exhibiting the most remarkable coincidence between theory and practice.

The importance of these results lies in their proving that in the matter of sand-filtration we are no longer working in the dark, but that we now know the factors upon which the success of the process depends, and by attention to which its efficiency may be maintained or even increased.

A searching investigation has recently been further made by one of us into the efficiency of the process of sand-filtration as practised by the several London water companies, the performance of the individual filter-beds having been, as far as possible, made the subject of examination. The results obtained are collected in the following tables[1]:—

Grand Junction Waterworks, Hampton, June 25, 1892
(Percy Frankland)

DESCRIPTION

Unfiltered Water

	No. of Colonies from 1 c.c. of water
First small storage reservoir nearest river . . .	1,703
Second small storage reservoir further from the river .	1,156
(The water does not come to rest in these small reservoirs, but passes through them to the filter-beds.)	
Intake from Thames	1,991

[1] Evidence given by Percy Frankland before the Royal Commission on Metropolitan Water-supply, November, 1892.

	No. of Colonies from 1 c.c. of water
Large storage reservoir; the greater part of its contents had been stored for six months, and none less than one month (leeward side) . . .	464
Ditto (windward side)	368
Old East Filter Bed	1,178
Old Middle „	1,627
Old West „	1,413
New East „	1,776
Filtered Water	
General Filter Well	95
„ „	80
„ „	80
Average =	85

Grand Junction Waterworks, Hampton, August 13, 1892

Filtered Water

Filter Bed	Age of Filter Beds in days	No. of Colonies from 1 c.c. of water
Old East	35 . . .	25
Old Middle	22 . . .	19
Old West	17 . . .	17
New West	12 . . .	23
General Filter Well . .	— . . .	23
Unfiltered Water		
Old East Bed . . .	— . . .	1,008
Old Middle Bed . . .	— . . .	1,029
Old West „ . . .	— . . .	1,222
New West „ . . .	— . . .	288

On June 25 the examination of the water on the several filterbeds, four in number, showed that this contained about the same number of bacteria as that in the small storage reservoir from which the filters were being supplied, and rather less than that in the Thames water at the intake itself. Unfortunately samples of the filtered water as it issues from the individual beds could not on this occasion be obtained, but only from the well in which the filtered water from the several beds collects. This filter well is, however, in immediate communication with a small service reservoir, so that the water in it is not necessarily wholly coming from the filters directly, but may be more or less

mixed with water which has been stored for a longer or shorter period in this service reservoir. Notwithstanding this disturbing factor, the contrast between the water before and after filtration is sufficiently marked. The average number of micro-organisms found in the unfiltered water taken from the surface of the four filter beds amounted to 1,498 per c.c., whilst that in the water from the filter well was only 85 in the same volume.

On August 13, 1892, the unfiltered water contained rather less bacteria than on the previous occasion, and that on the New West filter bed contained markedly less than any of the others, which is probably to be accounted for by the fact that the water in this filter bed was in a more quiescent state than that in the others, in consequence of the unfiltered water having to pass through the other beds before reaching this one, which forms as it were the 'dead end' of the system, and in this manner it furnishes further evidence of the removal of micro-organisms through subsidence. The average number in the unfiltered water was 887 per c.c. Samples were taken of the filtered water issuing from each of the four filter beds, and in each only a very small number of micro-organisms was found (maximum 25, minimum 17, average 21). These filters had been working from twelve to thirty-five days since being last cleaned, but the figures show that there was no difference in their efficiency. The number of micro-organisms in the water of the general filter well was also practically the same as in that from the individual beds, and this was to be anticipated, as I was informed by the resident engineer, Mr. Loam, that the tendency at the time would be rather for water to pass from the filter well into the small service reservoir than in the opposite direction.

West Middlesex Waterworks, Barnes, August 12, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
No. 1	14	98
No. 11	33	65
No. 2	62	25
No. 3	36	16
No. 9	3	27
No. 7	45	17
No. 10	10	22
No. 8	33	18

Unfiltered Water

Filter Bed	No. of Colonies from 1 c.c of Water	Filter Bed	No. of Colonies from 1 c.c of Water
No. 1	271	No. 3	288
No. 11	554	No. 7	691
No. 2	851	No. 10	447
No. 9	443	No. 8	308

West Middlesex Waterworks, Barnes, Oct. 3, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
Nos. 1 and 11 (principally from No. 11)	65 and 15	18
No. 2	47	11
No. 3	15	11
No. 5	50	12
No. 8	31	4
No. 9	55	8
		Average = 11

Unfiltered Water

Unfiltered Thames Water from Hampton	1,437
Unfiltered Thames Water which had only passed through one subsiding reservoir	318
Ditto, which had passed through two subsiding reservoirs	177

Thus in the experiments made at these works on August 12, 1892, the unfiltered and filtered waters of eight filter beds were submitted to examination.

The number of bacteria in the unfiltered water varied from 851 to 271, and averaged 482 in one cubic

centimetre, whilst the number in the filtered water ranged from 98 to 16, and averaged 36 in the same volume. The length of time which had elapsed since last cleaning varied between 3 and 62 days, but there was no connection discoverable between the age of the filters and efficiency of the filtration.

On October 3, 1892, the unfiltered water before and after storage, as well as the filtered water from seven filter beds, was submitted to examination.

Inasmuch as all the filter beds are supplied with water which has passed through at least one storage reservoir, and in some cases two, the number of micro-organisms in the water actually undergoing filtration may be taken as from 177 to 318 per cubic centimetre, whilst the numbers in the filtered water ranged from 4 to 18 in the same volume and averaged 11. The ages of the filter beds varied from 15 to 65 days, but in no case was the efficiency affected by the age of the bed.

Southwark and Vauxhall Waterworks, Battersea, August 19, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
Nos. 3, 4, 5	Cleaned 14 days before, charged since, but only began filtering 6 hours previously	70
No. 6	21	3
From main	—	31

Unfiltered Water

No. 3 Bed, representing Nos. 3, 4, and 5 Beds	394
No. 6 Bed	346

Unfortunately these works are ill adapted for an investigation of this kind, as the water coming from the individual filter beds is not in most cases accessible.

The first sample represents the united filtrate of three beds which had only been in operation six hours

previously; the second sample was taken from the filtrate of a bed which had been at work for twenty-one days; and the third sample was taken from the main at the works, and is thus representative of the united waters after filtration.

Samples of unfiltered water were also taken from the surface of the same filter beds; the number of micro-organisms found was very small for unfiltered Thames water, and thus again points to the removal of micro-organisms by subsidence, to which we have already referred.

Chelsea Waterworks, Thames Ditton, August 20, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
No. 4	68	13
No. 5	40	6
No. 3	54	27
No. 6	13	10
No. 1	7	21
No. 2	26	20
No. 7	76	24

Unfiltered Water

No. of Filter Bed	No. of Colonies from 1 c.c. of Water	No. of Filter Bed	No. of Colonies from 1 c.c. of Water
No. 4	418	No. 1	244
No. 5	855	No. 2	2,548
No. 3	381	No. 7	751
No. 6	345		

Thus the water from seven filter beds was examined, and the series is particularly interesting in consequence of the great length of time (76, 68, 54, and 40 days) during which some of these beds had been at work. There was no indication of their efficiency having become impaired through this prolonged service. The number of micro-organisms in the filtered water varied from 6 to 27, and averaged 17 in the cubic centimetre. The

unfiltered water in the several beds exhibited considerable variations as regards the number of micro-organisms, the maximum being 2,548, the minimum 244, and the average 792 per cubic centimetre.

Lambeth Waterworks, Thames Ditton, August 26, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
Old Works:		
No. 1	35	92
No. 2	21	36
No. 3	4	35
No. 4	17	41
New Works:		
No. 5	28	8
No. 6	32	11
No. 7	13	9
No. 8	2	12
No.	29	10
No. 10	16	13

Unfiltered Water

Filter Bed	No. of Colonies from 1 c.c of Water	Filter Bed	No. of Colonies from 1 c.c. of Water
Old Works:		New Works:	
No. 1	1,904	No. 5	1,217
No. 2	1,521	No. 6	1,108
No. 3	825	No. 7	1,118
No. 4	1,452	No. 8	Lost
Unfiltered Thames water from suction tank	1,392	No. 9	Lost
		No. 10	968

Lambeth Waterworks, Thames Ditton, September 30, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
New Works:		
No. 10	3	50
No. 9	24	23
No. 8	36	10
No. 7	10	8
No. 6	28	4
No. 5	22	6
General filter well of New Works	—	9

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of water
Old Works:		
No. 1	undergoing cleansing at the time	—
No. 2	17	32
No. 3	7	79
No. 4	14	56

Unfiltered Water

Unfiltered Thames water from suction tank	6,821
Do. do.	7,104

These works are particularly well adapted for such an inquiry as this, since each individual filter bed has a separate well into which the filtered water issuing from it passes. There are four filters at the so-called 'old' works and six at the new works on the other side of Portsmouth Road, and it is worthy of note that the number of micro-organisms found in the water of the old was distinctly greater than in that of the new works. It should be mentioned, however, that the sample from No. 1 filter was not a very satisfactory one, as the water in the well was rather low at the time and the bottom was touched in collecting the sample, and we are, therefore, of opinion that the result should be discarded. The series is particularly interesting in consequence of the different ages of the filters; thus No. 8 had only been at work for two days, whilst No. 6 had been at work for 32 days, and yet both were yielding a filtrate containing an equally small number of micro-organisms. The average number of micro-organisms in the filtered waters was 27, or, excluding that from No. 1 filter, in which the disturbance occurred during the collection of the sample, only 19 per cubic centimetre. The average number of micro-organisms found in the unfiltered water on the filter beds amounted to 1,264 per cubic centimetre.

On the second occasion the filtered water of the old

works again exhibited distinctly more micro-organisms than that of the new. The average number found in the filtered water taken directly from the beds amounted to 30 per cubic centimetre, and thus hardly differed from that on the previous occasion. On the other hand, the unfiltered Thames water yielded about 7,000 colonies per cubic centimetre as against about 1400 before, and this clearly shows that when such small numbers are found in the filtered water hardly any of them can be derived from the unfiltered water, but that they are probably mostly due to the fact that the filtering materials themselves contain micro-organisms, as do also the culverts and wells into which the filtered water passes; in fact the micro-organisms found in the filtered water are probably almost entirely due to post-filtration sources.

New River Waterworks, Stoke Newington, August 27, 1892

Filtered Water

No. of Filter Bed	Age of Filter Bed in days	No. of Colonies from 1 c.c. of Water
No. 1	1	154
No. 2	8	16
No. 3	10	18
No. 4	5	10
No. 5	23	9
General Filter Well	—	10

Unfiltered Water.

Water supplying Filters Nos. 1 and 2, 8 and 9	260
" " " Nos. 3, 4, 5, 6, and 7	171
New River Cut above Reservoir	677
Outlet of First Reservoir	560
Outlet of Second Reservoir	183

The water issuing from only five out of the nine filter beds was accessible. In all cases excepting one the number of micro-organisms found in the filtered water was very small, and it is particularly noteworthy that the filter bed yielding the water with the larger

number of micro-organisms had only been in operation for one day, showing that it had not yet settled down into the normal condition of efficiency which characterised the other beds. The number of micro-organisms in the united water from all the filters was only 10 per cubic centimetre. The figures also show the remarkable diminution in the number of micro-organisms in the unfiltered water by passing through the two large storage reservoirs.

East London Waterworks, Lea Bridge, September 2, 1892

Filtered Water

Description	No. of Colonies from 1 c.c. of Water
'Essex' Well from 6 filters . . .	30
'New' Well from 12 filters . . .	12
'Middlesex' Well from 6 filters . .	12

Unfiltered Water

From the 'feeder' supplying the filter beds	676

There are no less than 24 filter beds at these works, but they are unfortunately so arranged that it is impossible to obtain samples representative of the water coming from any one of them singly. Samples were taken from the three wells, in each of which the water from a group of filters is collected. The average number of micro-organisms in the filtered water was very small, and amounted to only 18 per cubic centimetre. The unfiltered water supplying the filter beds contained 676 in the same volume. This unfiltered water was derived from the large storage reservoirs at Walthamstow, and the comparatively small number of micro-organisms which it exhibits shows that a very considerable reduction in the suspended bacteria must have taken place during the storage in those reservoirs, for the water of the Lea itself at the intake must cer-

tainly have contained much more than 676 micro-organisms per cubic centimetre.

Summary.—The results arrived at in this further investigation may be summarised as follows :—

(1) That there is a most striking reduction in the number of suspended micro-organisms during storage in large reservoirs. The strongest evidence of this was afforded in the case of the large reservoir of the Grand Junction Company at Hampton, the storage reservoirs of the West Middlesex Company at Barnes, and those of the New River Company at Stoke Newington.

(2) The most searching examination which has yet been instituted into the efficiency of the filtration carried out by the London water companies shows that out of 61 samples of filtered water collected at the several works only one sample yielded more than 100 colonies per cubic centimetre, whilst the average number was only 29.

(3) The average numbers in the filtered waters of the several companies were as follows :—

New River (excluding one exceptional sample) .	13
Chelsea	17
East London	18
West Middlesex	25
Lambeth	27
Southwark	35
Grand Junction (excluding the samples from the general filter well)	21

These numbers show how very uniform, from a bacteriological point of view, was the filtered water produced by the several companies.

(4) In almost every case the number of micro-organisms in the filtered water appeared to be independent of the age of the filter bed, but only on two occasions (one at the New River, and one at the South-

wark works) were filter beds examined on the first day after cleaning, and on both of these occasions there was evidence that the water issuing from such new beds was not as free from micro-organisms as that coming from older beds at the same works. It appears, however, from the results obtained with beds which had been recently cleaned that normal efficiency is attained within two or three days. On the other hand, there was no evidence of the efficiency of beds being reduced through prolonged use.

(5) Of the small number of micro-organisms found in these filtered waters, it appears that only a very insignificant part of them can be derived from the unfiltered water, for the numbers in the filtered bear no relationship to those in the unfiltered waters; the majority of the bacteria in the filtered water are, therefore, attributable to post-filtration sources. It must be remembered in this connection that the filtering materials are not sterile themselves, nor are the channels, culverts, well, &c., into which the filtered water passes.

Berlin Water Supply, River Spree and Lake Tegel.—The water supply of Berlin is derived from the River Spree and Lake Tegel, both of which are submitted to sand-filtration before distribution. Plagge and Proskauer, Wolffhügel and others, have made a large number of examinations to ascertain the bacterial contents of the water supplied from these two sources before and after filtration.

The following table furnished by Plagge and Proskauer [1] shows the reduction in the number of micro-organisms effected by the treatment to which the water has been subjected at the waterworks.

[1] *Zeitschrift für Hygiene*, vol. ii. 1887, p. 401.

Berlin Water Supply before and after Sand-Filtration (Plagge and Proskauer)

Number of Micro-organisms obtained from 1 *c.c. of Water*

Date	River Spree before filtration	River Spree after filtration	Date	Lake Tegel before filtration	Lake Tegel after filtration
June 2, 1885 .	5,475	42	June 2, 1885 .	118	16
„ 9 „	7,980	22	„ 9 „	117	39
„ 16 „	6,100	33	„ 16 „	115	76
„ 23 „	6,100	41	„ 23 „	1,325	194
„ 30 „	4,400	53	„ 30 „	880	44
July 7 „	3,500	28	July 7 „	—	42
„ 14 „	7,200	200	„ 14 „	1,896	120
„ 21 „	110,740	1,656	„ 21 „	13,220	49
„ 28 „	2,640	54	„ 28 „	1,500	48
Aug. 4 „	2,310	70	Aug. 4 „	900	28
„ 11 „	3,600	65	„ 11 „	1,100	434
„ 18 „	1,800	36	„ 18 „	179	50
„ 25 „	11,900	26	„ 25 „	4,410	21
Sept. 1 „	3,360	184	Sept. 1 „	600	17
„ 8 „	960	1,000	„ 8 „	1,220	100
„ 15 „	4,500	44	„ 15 „	158	56
„ 22 „	9,200	44	„ 22 „	130	55
„ 29 „	1,120	30	„ 29 „	111	31
Oct. 6 „	3,192	36	Oct. 6 „	160	24
„ 13 „	1,204	25	„ 13 „	519	29
„ 20 „	2,178	36	„ 20 „	174	18
„ 27 „	4,840	24	„ 27 „	173	10
Nov. 3 „	8,500	80	Nov. 3 „	128	82
„ 10 „	2,520	42	„ 10 „	250	32
„ 17 „	6,000	52	„ 17 „	60	51
„ 24 „	31,500	167	„ 24 „	251	78
Dec. 1 „	9,000	117	Dec. 1 „	65	10
„ 8 „	2,700	220	„ 8 „	240	210
„ 15 „	5,880	180	„ 15 „	1,290	1,500
„ 22 „	5,600	34	„ 22 „	86	260
„ 29 „	4,000	20	„ 29 „	149	110
Jan. 5, 1886 .	4,500	95	Jan. 5, 1886 .	80	38
„ 12 „	1,400	40	„ 12 „	170	12
„ 19 „	1,100	94	„ 19 „	92	36
„ 26 „	29,000	100	„ 26 „	54	60
Feb. 2 „	20,000	80	Feb. 2 „	13,600	24
„ 9 „	5,900	7	„ 9 „	15	6
„ 16 „	1,250	10	„ 16 „	30	2
„ 23 „	1,280	8	„ 23 „	14	8
March 2 „	1,010	8	March 2 „	57	3
„ 9 „	3,680	112	„ 9 „	225	19
„ 16 „	14,400	210	„ 16 „	440	70
„ 23 „	32,700	145	„ 23 „	16,500	66
„ 30 „	100,000	2,300	„ 30 „	50,000	104

Lake of Zürich.—It will be interesting to compare

the results of sand-filtration on river waters containing a large number of micro-organisms with those obtained when microbially very pure water has to be dealt with. For this purpose the water-supply of Zürich has been selected. The source from which it is derived is the Lake of Zürich, and before delivery it is subjected to sand-filtration.

In the report[1] on the water-supply of Zürich for 1889 details are given of the conditions under which some of the filters were working, and of the manner in which these conditions affected the microbial purity of the filtered water. The results are brought together in the following table:—

Special Investigations on the Working of Particular Filter Beds in Zürich from July 6 *to September* 7, 1889

Filter Beds

Condition	Covered			Open	
	Old	Sand renewed	Entirely new	Sand renewed	Old
	I.	II.	III.	IV.	V.
Age of working in months	42	6·6	(0·6)	7·5	30·8
Thickness of filtering sand in cm.	29	70	80	66	36
Bacteria per c.c.	29	12	6	12	12

Filters I., II., IV. and V. were old ones, having been constructed from two to four years previously; of these Nos. I. and V. had had the surface sand scraped off from time to time, and the residual layer of sand had thus become considerably reduced in thickness; Nos. II. and IV. had respectively had their fine sand completely renewed 6·6 and 7·5 months previously; whilst No. III. had been totally reconstructed from top to bottom 0·6 month before the above investigation was made. The results recorded are the means of eight

[1] *Jahresbericht über die Wasserversorgung von Zürich und Umgebung*, 1889.

separate determinations in each case. The rate of filtration was carefully regulated in each filter to a fall in level of 26 to 28 centimetres per hour.

The differences in the number of micro-organisms found are so small that it is impossible to draw any comparative conclusions from them; but it is evident that, at any rate with such pure water as was obtained from the Lake of Zürich, it is possible for sand filters to be kept working for from three to four years—which is the period given in the report—and yet for them to yield an exceedingly pure filtrate from a bacterial point of view; also that the simple renewal of the upper layers of sand is sufficient to ensure the efficient working of the filters.

In connection with the influence of the thickness of the layer of fine sand upon the filtrate, it is interesting to note that in the course of removing the 70 cm. layer of sand of one of the filter beds, which was yielding an excellent filtrate, it was found that when only 25 cm. was left the filtrate became unsatisfactory; in consequence of which it was decided not to reduce the layer to less than 30 cm., but when this limit was reached in the course of successive scraping, to commence the renewal of the sand.

Some very interesting differences in the working of covered and uncovered filter beds are also given; thus whilst the amount of water delivered by the former was on the average 1,479,000 cubic metres per annum, the latter yielded only 1,275,000 cubic metres, *i.e.* about 14 per cent. less, which must be attributed to their being rendered less pervious by the more rapid formation of slime through the growth of algae requiring daylight, and to the effect of frost. Thus the surfaces of the covered filters had on the average to be scraped seven

times, those of the uncovered ones nine times in the year; the average thickness of sand removed in scraping being 20 mm. each time.

On renewing the sand in a filter, it was found that it was only after from $3\frac{1}{2}$ to $36\frac{1}{2}$ days that it got into an efficient working condition and yielded a satisfactory filtrate. The time required for such conditioning of a filter is of course exceptionally great in a case like this, in which a comparatively clear water is being filtered.

In the following table the bacterial composition of the water is given before and after filtration for the year 1891 [1]:—

Unfiltered Water from the Lake of Zürich, 1891

Pumping Station

1891	Number of Micro-organisms per c.c.		
	Max.	Mean	Min.
Average for 1st quarter . . .	2,179	777	44
,, ,, 2nd ,, . . .	1,425	525	101
,, ,, 3rd ,, . . .	327	245	142
,, ,, 4th ,, . . .	833	384	184

Filtered Water taken from the Filtered Water Reservoir, 1891

1891	Number of Micro-organisms per c.c.		
	Max.	Mean	Min.
Average for 1st quarter . . .	27	10	2
,, ,, 2nd ,, . . .	22	10	1
,, ,, 3rd ,, . . .	27	12	3
,, ,, 4th ,, . . .	27	15	4

In consequence of the extreme cold the lake was frozen in this year, and some special investigations were made to ascertain the effect upon the microbial condi-

[1] 'Licht- und Wasserwerke Zürich.' *Jahresbericht für* 1891.

tion of the water. Chemically no difference could be detected, but bacterially the unfiltered water in February, March and April exhibited an abnormally large number of micro-organisms, causing the average for the year to rise from 123 in 1890 to 583 in 1891 per c.c. On the other hand, so excellent was the working of the filters that the filtrate was totally unaffected; the average for the filtered water in 1890 being 24, and in 1891 23 micro-organisms per c.c.

The report goes on further to state that, whilst in the year 1880, when the lake was frozen, an epidemic of diarrhœa and typhoid fever which occurred was attributed to the water-supply, in 1891, in spite of the long duration of the ice on the lake, the public health in these respects was not only normal, but if anything more satisfactory than usual. It has also been satisfactorily proved, says the report, by careful examination of statistics, that since the establishment of the new filtration-works in 1886 a very marked diminution has taken place in the number of cases of typhoid.

So far we have only had under consideration instances in which sand-filtration has been so efficiently carried out that the results are eminently satisfactory. But it must not be imagined that this is invariably the case, and the following instances are given to illustrate how dependent are the results obtained upon the care with which the process is conducted.

Königsberg Water-supply.— Königsberg[1] is supplied with water from springs, streams and wells. The main supply is obtained at some distance from the town, in

[1] 'Bericht über die bakteriologische Untersuchung des Königsberger Wasserleitungswasser in der Zeit von December 1890 bis December 1891,' Laser. *Centralblatt für allgemeine Gesundheitspflege*, 1892, p. 133.

the neighbourhood of the Alk mountains, from a stream which is dammed so as to form an artificial lake. The outflow from the latter is then united with another smaller stream, also some way out of Königsberg; these sources are then further connected with a third supply, which is derived partly from springs and partly from ground-water, the latter gaining access to the main, which is purposely interrupted at intervals in its course to admit of this augmentation of the supply taking place. The united waters are then led on to 5 covered sand filter beds, the filtrate from which is stored in reservoirs. The filter beds are constructed of a foundation consisting of a layer of large flints 20 cm. in depth, followed by a layer 10 cm. in height of finer flints about the size of hazel-nuts, upon which is placed a layer of 5 cm. of the same material, but not larger than peas, 5 cm. of the size of lentils, 5 cm. the size of pin-heads, and, finally, at the top there is a stratum of 65 cm. of sand. This sand was, however, when these investigations were made, very dirty, being mixed with soil, and, moreover, was not of a uniform degree of fineness. This, according to Laser, was sufficient to account for the large number of micro-organisms found on some occasions in the filtrate; but in addition to these unfavourable circumstances the filter beds were at times kept so long in use and became so choked, that a high pressure had to be exerted to force the water through.

The following table gives the date of the examination, the number of micro-organisms per c.c., the pressure, the number of days the filter had been working at the time of examination, and the length of time the unfiltered water had been allowed to remain on the filters before the latter were put to work.

Königsberg Water Supply (Laser)

Date	Unfiltered water	Filtered water	Pressure in mm.	Days of working	Days during which water remained on filter
	Number of Micro-organisms in 1 *c.c.*				
	FILTER I.				
Feb. 3, 1891 .	1,400	630	500	8	8
Oct. 9 ,,	2,916	1,080	100	1	4
Nov. 23 ,,	12,177	—	550	15	3
Dec. 7 ,,	7,290	819	100	1	13
,, 14 ,,	14,175	676	200	8	13
,, 23 ,,	11,187	—	600	17	13
	FILTER II.				
June 4 ,,	640	160	200	10	4
,, 26 ,,	700	70	400	31	4
July 11 ,,	—	30	500	12	5
,, 25 ,,	360	70	200	6	4
Aug. 14 ,,	720	398	100	1	3
,, 29 ,,	1,050	300	400	16	5
Sept. 25 ,,	6,048	2,024	300	21	3
Oct. 9 ,,	6,048	5,836	650	35	3
	FILTER III.				
July 11 ,,	680	70	450	27	0
Aug. 14 ,,	2,110	112	200	6	3
Sept. 25 ,,	5,184	1,304	200	17	5
Nov. 23 ,,	15,681	726	300	7	8
Dec. 7 ,,	11,340	2,630	600	21	8
,, 14 ,,	12,960	3,402	350	4	4
	FILTER IV.				
Dec. 11, 1890 .	352	270	400	4	1
March 2, 1891 .	980	350	200	4	4
April 6 ,,	800	300	600	6	5
July 11 ,,	800	180	200	1	4
,, 25 ,,	1,260	320	250	16	4
Aug. 29 ,,	700	432	700	26	2
Nov. 23 ,,	5,040	680	100	1	7
Dec. 14 ,,	11,340	2,816	600	23	7
,, 23 ,,	11,700	1,530	300	5	4
	FILTER V.				
Jan. 5 ,,	420	200	400	26	16
May 4 ,,	2,450	1,840	200	7	3
July 25 ,,	960	180	350	29	3
Aug. 29 ,,	1,340	128	350	12	4
Sept. 25 ,,	11,520	4,410	700	39	4
Oct. 9 ,,	10,804	6,720	250	9	6
Dec. 7 ,,	6,048	1,260	300	9	12
,, 23 ,,	13,230	871	150	1	3

It will be seen that the worst result was obtained with Filter II. on October 9, 1891. In this case there were 6,048 in the unfiltered water, and as many as 5,836 in the filtrate, and it must be noted that the pressure was as high as 650 mm., and the filter had been working as long as 35 days.

Again we find with Filter IV. an unsatisfactory reduction on August 29, 1891. The unfiltered water only contained 700, but the filtrate as many as 432. Here again the pressure was high, being 700 mm., and the filter had been working for 26 days. Similarly with Filter V. on January 5, 1891, the reduction was only 50 per cent., the pressure being 400 mm., and the filter working for 26 days. Again, with the same filter on September 25, an unsatisfactory reduction is observable; whilst in this case also the pressure was high, and the filter had been working a large number of days. There does not appear from the table to be any special connection between the filtrate and the number of days the water remained on the filters before being passed through.

The principal point which these results exhibit is the great reduction in the efficiency of filtration which ensues when the pressure employed is excessive, for from the results obtained in other places, notably in London, there can be no doubt that the greater age of the filters in which these higher pressures were used was not the factor determining the inefficiency, although the high pressures had, of course, to be employed in consequence of the age of the filters having impaired their perviousness. It is in this way that in actual practice the age of a filter may indirectly lead to inefficiency in filtration.

Paisley Water Supply.—The sand filters belonging to the waterworks of Paisley, N.B. have been recently

examined by one of us, and the results are of interest as exhibiting the manner in which defective filtration is detected by the bacteriological examination. The unfiltered water, which is of moorland origin, is derived from two different reservoirs (Rowbank and Camp Hill) of large size, and is submitted to filtration at an unusually high velocity, viz. about five gallons per square foot per hour. On the occasion in question all the unfiltered water was coming from the Camp Hill reservoir. The samples of filtered water were in every case taken directly from the filter wells of the individual beds, and the results obtained were as follows :—

		Number of Micro-organisms in 1. c.c.
Camp Hill Water (unfiltered)		3,414
Filter No. 1	Johnstone Works	181
„ 2		232
„ 3		197
„ 4	Stanley Works	74
„ 5		324
„ 6		566
„ 7		112
„ 8		42

It is particularly worthy of note that in the case of Filters Nos. 5, 6, and 7, the unfiltered water was visibly leaking through the retaining walls of the filter beds into the filter wells, and two of these, it will be seen, exhibited a particularly large number of bacteria.

If the above results be compared with those already recorded for the London and Zürich waterworks, it will be seen how greatly inferior is the efficiency of the process of sand-filtration as practised at Paisley and Königsberg.

These investigations clearly show that sand-filtration, when carefully pursued, offers a most remarkable and obstinate barrier to the passage of microbes; and there is, as already pointed out, every justification in presuming

that if disease organisms are at any time present in the raw untreated water they will be similarly retained, as there is no reasonable ground for supposing that they should behave differently in this respect from the ordinary harmless water bacteria. That this is no mere hypothesis but an actual fact has been demonstrated in the most conclusive manner by the experience recently gained in Hamburg and Altona respectively during the cholera epidemic of 1892.[1]

Hamburg and Altona Water-supplies.—These two cities are both dependent upon the river Elbe for their water-supply, but whereas in the case of Hamburg the intake is situated *above* the city, the supply for Altona is abstracted below Hamburg *after it has received the sewage of a population of close upon* 800,000 *persons.* The Hamburg water was, therefore, to start with, relatively pure when compared with that destined for the use of Altona. But what was the fate of these two towns as regards cholera? Situated side by side, absolutely contiguous in fact, with nothing in their surroundings or in the nature of their population to especially distinguish them, in the one cholera swept away thousands, whilst in the other the scourge was scarcely felt; in Hamburg the deaths from cholera amounted to 1,250 per 100,000, and in Altona to but 221 per 100,000 of the population. So clearly defined, moreover, was the path pursued by the cholera, that although it pushed from the Hamburg side right up to the boundary line between the two cities, it there stopped, this being so striking that in one street, which for some distance marks the division between these towns, the Hamburg side was stricken down with

[1] 'Wasserfiltration und Cholera,' Koch. *Zeitschrift für Hygiene*, vol. xiv. p. 393, 1893. 'Die Cholera in Deutschland während des Winters 1892 bis 1893,' Koch, *ibid.*, vol. xv. p. 89, 1893.

cholera, whilst that belonging to Altona remained free. The remarkable fact was brought to light that, in those houses supplied with the Hamburg water, cholera was rampant, whilst in those on the Altona side and furnished with the Altona water not one case occurred.

The Hamburg water, however, to start with was, as we have seen, relatively pure when compared with the foul liquid abstracted from the Elbe by Altona; but, whereas in the one case the water was submitted to exhaustive and careful filtration through sand before delivery, in Hamburg the Elbe water was distributed in its raw condition as taken from the river. But further testimony was afforded later to the truth of this interpretation; for during the ensuing winter, when the cases of cholera had almost completely died out in Hamburg, suddenly a most unexpected and unaccountable recrudescence of the epidemic occurred, and this time in Altona. This outbreak could not be traced to any direct infection from Hamburg, but must have arisen in Altona itself. In all, about forty-seven cases were recorded between December 23, 1892, and February 12, 1893. A searching inquiry was instituted, and it was ascertained that the number of bacteria found in the filtered water, usually about 50, had during these months risen to as many as 1,000 and more in 1 c.c., clearly indicating that the filtration of the water was not being efficiently carried out. That this was actually the case was proved by the fact that one of the sand filters, which had been cleaned during the frost, had become frozen over, and was in consequence not able to retain the bacteria. That the outbreak did not become more serious Koch ascribes to the fact that this imperfectly filtered water was so largely diluted by that which had been efficiently filtered.

There cannot be any longer a doubt, therefore, as

to the value of sand-filtration as a means of water purification; but the responsibility which we have seen attaches to this treatment of water cannot be exaggerated, for whilst when efficiently pursued it forms a most important barrier to the dissemination of disease-germs, the slightest imperfection in its manipulation is a constant menace during any epidemic.

It will be necessary now to turn our attention to those investigations of practical interest which have been carried out on an experimental scale.

Experimental Sand-Filtration.—Fraenkl and Piefke[1] studied the question of sand-filtration by taking the Berlin water and passing it through specially constructed sand filters, which were arranged so as to imitate as nearly as possible in miniature the process of filtration as conducted on the large scale at the water-works. Their object was to trace, if possible, the effect of varying the several conditions under which filtration is carried out upon the number of micro-organisms in the filtered water.

In the first instance filtered Berlin water and new sand were used. In order to find out whether the organisms contained in the water supplied to the filter were to be found in the filtrate, they purposely introduced large numbers of the *Bacillus violaceus* (see p. 472) into the unfiltered water. This is a non-pathogenic organism, found only very occasionally in water; it produces a violet pigment in the media in which it is cultivated, and hence is readily recognisable amongst other organisms on a gelatine-plate. Two filters were used, one (A) allowing of a rapid (300 mm. in an hour), and the other (B) of a much slower rate of filtration (100 mm. in the hour). It was found that the difference in the rate of filtration produced a marked

[1] *Zeitschrift für Hygiene*, vol. viii. 1890, p. 1.

effect upon the two filters: that, whereas (A), after thirty days' work, was practically useless, owing to its having become clogged up; (B) was hardly affected at all, and could have been kept in operation for weeks longer. Further, that the number of micro-organisms in the filtrate bore a distinct relationship to the rate at which the filtration took place, for the number of *B. violaceus* colonies was in every case larger in the filtered water from (A) than in that from (B). Taking the results of observations extending over a whole month, the number of *B. violaceus* colonies was found for (A) to be 894, for (B) 361 in the c.c. Again, in another series of experiments carried on simultaneously the number for (A) was 1,578, for (B) 607. Another series of experiments was carried on with filters in which the sand was not fresh, but taken from a real filter bed which had been some time in use, and the water employed was the raw river-water. These materials were selected in order to procure the slimy deposit which forms on the surface of sand filters, and the raw river-water was substituted for the filtered in order, by providing more organic material, to stimulate the formation of this slime. The rate of filtration for the two filters (A and B) was kept respectively at 300 mm. and 50 mm. per hour. *Bacillus violaceus* was added to the unfiltered water as in the previous experiment.

Here again it was found that in the filtrate from (A) nearly six times as many *violaceus* colonies were obtained as in that from (B), and the more rapid rate of filtration so completely clogged (A) that after eight days it had to be stopped and cleaned, whilst (B) went on working for thirty days. It is, moreover, particularly noteworthy that the numbers of bacteria found in the filtered water were markedly less in this second set of filters (A and B) than in the first, although the number of bacteria in the

Experimental Filtration of River Spree Water (C. Fränkel and Piefke)

Date	Unfiltered water. Number of bacteria in 1 c.c.		Filter A				Filter B				Daily yield of filters in litres	
			Filtered water. Number of bacteria in 1 c.c.		Rate of filtration per hour	Pressure employed	Filtered water. Number of bacteria in 1 c.c.		Rate of filtration per hour	Pressure employed		
	Total number found	Number of B. violaceus found	Total number found	Number of B. violaceus found	mm.	mm.	Total number found	Number of B. violaceus found	mm	mm.	A	B
July 5, 1889	—	—	—	—	300	180	—	—	50	37	2,880	480
„ 6 „	—	—	620	60	300	135	580	61	50	32	„	„
„ 7 „	—	—	320	80	300	211	240	40	50	44	„	„
„ 8 „	Innumerable	Innumerable	420	40	300	344	?	28	50	59	„	„
„ 9 „	„	„	130	18	300	402	110	4	50	57	„	„
„ 10 „	„	„	270	30	300	588	16	10	50	59	„	„
„ 11 „	„	„	110	1	300	710	48	5	50	60	„	„
„ 12 „	„	„	156	21	300	1,048	92	1	50	63	„	„
			The filter was cleaned									
„ 13 „	—	—	—	—	300	124	130	0	50	63	—	„
„ 14 „	Innumerable	Innumerable	1,390	130	300	212	26	0	50	65	2,880	„
„ 15 „	„	„	240	50	300	340	14	0	50	64	„	„
„ 16 „	„	„	460	90	300	400	14	2	50	78	„	„
„ 17 „	„	„	520	60	300	584	13	3	50	89	„	„
„ 18 „	„	„	420	26	300	787	129	3	50	102	„	„
„ 19 „	„	„	320	40	300	840	286	2	50	120	„	„
„ 20 „	„	„	160	28	300	1,048	31	3	50	178	„	„
			The filter was cleaned									
„ 21 „	—	—	—	112	300	—	114	1	50	210	—	„
„ 22 „	Innumerable	Innumerable	560	116	300	126	230	1	50	224	2,880	„
„ 23 „	„	„	220	40	300	198	116	1	50	236	„	„
„ 24 „	„	„	160	26	300	335	230	2	50	258	„	„
„ 25 „	„	„	110	22	300	396	61	2	50	295	„	„
„ 26 „	„	„	210	15	300	467	260	1	50	350	„	„
„ 27 „	„	„	62	8	300	588	220	1	50	397	„	„
„ 28 „	„	„	96	12	300	700	210	1	50	490	„	„
„ 29 „	„	„	31	5	300	834	60	4	50	600	„	„
„ 30 „	„	„	110	4	300	968	43	14	50	720	„	„
„ 31 „	„	„	90	15	300	1,060	96	21	50	810	„	„
Aug. 1 „	„	„	90	6	300	1,060	107	20	50	968	„	„
			The filter was cleaned									
„ 2 „	—	—	—	6	300	—	—	34	50	1,060	—	„
Sum for the whole month . .			—	1,061	—	—	—	265	—	—	74,880	13,920

unfiltered water was much greater in this second series of experiments than in the first. The greater efficiency manifested by this second series of filters is attributable to the more thorough coating of the sand with a stratum of slime.

The careful and constant testing of these experimental filters showed, what has been demonstrated by one of us on the large scale in the case of the London filter beds (see p. 139, New River table), that the water which first passes through the filter is but slightly affected, only little reduction taking place in the number of micro-organisms, and that it is only after the filter has been at least one day in use that the full effect is obtained.

These experiments also conclusively establish that the rate of filtration is the factor of commanding importance; for by increasing the rate not only is the filtrate deteriorated, but the filter becomes quickly clogged, necessitating constant renewal, which, seeing that it does not attain its normal efficiency at once, must necessarily reduce the reliability placed in the purifying power of sand-filtration.

It will, however, be seen from the table (p. 156) that the quick filter (A) had in eight days, when cleaning was first necessary, passed much more water than the slow filter in the course of the whole month, so that with an insufficient area for filtration it is absolutely necessary to employ high pressures, rapid filtration, and frequent cleaning, leading of course to imperfect bacterial purification.

One of the most important results arrived at in these experiments of Fraenkel and Piefke is the proof which they furnish that the sand filters, even under the most favourable conditions of working, do not form a complete obstacle to the passage of micro-organisms. Thus, even when the rate of filtration was reduced to a

minimum (25 mm. per hour), some, although very few, colonies of *Bacillus violaceus* were obtained from the filtrate. Again, in similar experiments in which cholera and typhoid bacilli were purposely added to the unfiltered water, these were also met with, in greatly diminished numbers of course, in the filtrate; and it is worthy of note that the cholera appeared to be more completely retained by the filter than the typhoid bacilli.

Piefke,[1] who is the resident engineer at the Berlin waterworks, has made some instructive investigations as to the manner in which the coating of slime on the filter beds affects the results of filtration.

A kilogram of sand taken from one of the Berlin filter beds was found when examined from the surface to contain 5,028 millions of bacteria, about 2 cm. deeper 734 millions, 10 cm. 190 millions, 20 cm. 150 millions, and 30 cm. 92 millions; whilst the layer of fine flints upon which the sand rests contained 68 millions. These examinations show that the upper layers of slimy sand afford a very important barrier to the passage of micro-organisms. When a filter starts working the micro-organisms present in the filtering material and introduced in the water are partially washed through; those which remain behind become attached to the sand particles, multiply and produce the well-known slimy coat.

These multiplying centres of bacteria, which at first are more especially found in the upper layers of the filter, serve as points of attraction and adhesion for those bacteria which have not been retained by the slime. This coating of slime gradually then spreads to the lower layers of the sand, which becomes slimy to the touch, losing all feeling of sharpness, throughout the

[1] *Die Principien der Reinwassergewinnung vermittelst Filtration.* Berlin, 1887.

entire mass, although the actual discoloration of the sand is only apparent in the first, or at most second, cm.

In a small experimental filter constructed by Piefke with *sterilised* waterworks sand, and set to work in the usual way, it was found that the power of retaining microbes was *nil*, that in fact more organisms were often found in the filtrate than in the unfiltered water, multiplication having taken place during passage through the filter. These experiments with sterilised sand thus clearly demonstrate that it is the slime deposit on the sand which constitutes the real filtering material in the waterworks filter.

It used to be a doctrine very generally enunciated by the German school of investigators, that these ordinary sand filters were endowed with the almost miraculous power of arresting *all* micro-organisms in the water passing through them, the presence of the microbes actually found in the filtered water being attributed *entirely* to post-filtration sources. Now, although in some individual filter beds, when the rate of filtration is well chosen and carefully regulated, the number of microbes is actually reduced almost to *nil*, as seen from the investigations conducted by one of us on the individual London water companies' filter beds; yet we have from the very first pointed out that these sand filters can only lay claim to relative and not to absolute efficiency, and the experiments we have been considering abundantly prove the correctness of our conclusions and deductions, which are now shared by our neighbours on the Continent.[1]

Purification of Sewage by Sand-Filtration.—We have so far only considered the purification of water by

[1] 'The Hygienic Value of the Bacteriological Examination of Water,' Percy Frankland. *Transactions of the International Congress of Hygiene and Demography*, vol. ii. p. 291. London, 1891.

means of sand-filtration, but its application to sewage has been made the subject of most exhaustive investigations in America. The experiments conducted by the State Board of Health of Massachusetts [1] form a classical piece of work on the question of sewage purification by intermittent filtration and chemical precipitation; but it is only possible here to indicate the lines upon which these researches were undertaken, and to give a brief summary of the results obtained.

Experimental tanks were placed in the open and under cover, the former were made of cypress, circular in plan, sixteen feet eight inches in diameter inside at the bottom, seventeen feet four inches at the top, and six feet deep inside. The tanks were made completely water-tight, and in each an under-drain fifteen feet in length, of horse-shoe section of about two square inches in area, was placed with open part downwards, and half an inch above the bottom, resting on blocks six inches apart; the floor of the tank was covered with one layer of coarse gravel stones about one inch by two inches, this by another layer of smaller size, upon which followed successive layers of gravel, the particles of which diminished in size to one-eighth of an inch in diameter, the thickness of the stratum of gravel being three and a half inches. This fine gravel was covered with a layer of very coarse mortar-sand, three and a half inches deep in the middle of the tank. This substratum, as described above, was the same for all the tanks, whilst to each tank was further added a special layer at the top consisting respectively of different kinds of sand, peat, river silt, brown soil, &c., the filtering

[1] *Experimental Investigations by the State Board of Health of Massachusetts upon the Purification of Sewage*, 1888-1890, Part II. Boston, 1890. The Twenty-fourth Annual Report of this Board (1893) also contains a very large number of further investigations on both the purification of sewage and water by filtration.

value of which it was desired to compare. The tanks within the building, on the other hand, were of galvanized iron, of about the same depth as the larger wooden tanks, but with only a diameter of twenty inches, giving an area of surface one-hundredth of that of the large tanks, or one twenty-thousandth of an acre. They were all provided with a substratum similar to that described for the large tanks, and were filled with various depths and varieties of materials. The sewage supplied to the tanks was abstracted from the main sewer of the city of St. Lawrence, about one thousand feet above its outlet, and above the entrance of streams from the manufacturing establishments, and may be regarded, therefore, as typical city sewage obtained from the shops and dwellings of perhaps ten thousand persons. It must be borne in mind, however, that American sewage is much weaker than that with which we are accustomed to deal, it being largely diluted with pure water. An American sewage stronger even than usual would contain as much as 998 parts of pure water per 1,000.

It would be impossible here to enter into the details of the experiments made upon each individual tank, but we have selected one of the large tanks as an illustration of the scale upon which the investigations were made, whilst the general results obtained with the different tanks will be shortly summarized.

Tank No I. then, after being packed with the substratum of material previously described, was filled five feet in depth with very coarse, clean mortar-sand, the total amount, including what was used in the substratum, being about 9,000 gallons. Sewage was first applied intermittently on January 10, 1888. Previous to that time water had been passing through the filter at the rate of 1,000 gallons a day for a month. The

filtration was much interfered with by snow and ice; on one occasion a depth of snow and ice of one foot was found above the sand, and ten inches of frost within the sand; but although the latter was so frozen that no opening could be found for a fine steel point, yet it nevertheless allowed liquid to pass through it.

The table on following page shows the number of micro-organisms found in a c.c. of the sewage applied, and in the effluent from January 3, 1888, when the tank was first set to work, to October 25, 1889, when the experiments were stopped.

The quantity of sewage passing through the filter in the four months previous to June 18, 1888, was at the rate of about 30,000 gallons per acre per day, except during a part of March, when a larger quantity was flowing. After June 18, 1888, through October, 1889, the daily quantity was nearly double, averaging 58,000 gallons per acre per day.

On August 21, 1888, a slight deposit of organic matter was observed on the surface, but after a week this disappeared, and then the only change apparent in the surface, from the time when sewage was first applied, was the growth of some small tufts of grass. Although the sewage was distributed over the whole surface with care, it was found that in September and October 1888 the greater part of the sewage was flowing to spots on the surface which were lower than the remaining area, and having become choked allowed less sewage to pass, causing a delay in its disappearance. Slight inequalities in the surface were removed by lightly raking over about one-half the area of the tank, and the tufts of grass were pulled up. A contrivance was also arranged to ensure an exact distribution of the sewage over the whole area, and the surface, with the slight exceptions mentioned above, remained untouched

Number of Bacteria found in 1 *c.c. of Sewage applied to and in the Effluent derived from Tank No.* 1 *from January* 1888 *until October* 1889

Date	Number of bacteria per c.c.		Date	Number of bacteria per c.c.		Date	Number of bacteria per c.c.	
	Sewage	Effluent		Sewage	Effluent		Sewage	Effluent
1888			1888			1888		
Jan. 3	—	27	June 7	1,260,000	—	Dec. 21	409,500	27
„ 5	—	36	„ 9	—	3,168	„ 26	415,800	8,232
„ 7	—	43	„ 14	—	528			
„ 10	897,611	14	„ 16	1,584,000	—	1889		
„ 12	1,071,048	9,094	„ 21	—	8,400	Jan. 2	355,000	11,020
„ 14	873,000	42,170	July 3	—	4,928	„ 8	518,800	1,306
„ 17	765,000	135,900	„ 13	—	5,886	„ 15	228,900	8,910
„ 19	1,512,000	222,234	„ 17	—	4,230	„ 22	2,038,400	41
„ 21	1,848,000	387,600	„ 21	—	3,600	„ 29	305,600	2,820
„ 24	126,000	86,490	Aug. 7	—	38	Feb. 6	173,950	9,828
„ 26	1,050,000	170,080	„ 16	—	72	„ 12	134,600	2,516
„ 28	929,250	15,246	„ 21	—	63	„ 19	137,550	30
„ 31	1,134,000	12,315	Sept. 11	—	2,508	„ 27	102,400	4,890
Feb. 2	211,200	2,119	„ 15	—	113	Mar. 5	126,300	5,075
„ 4	258,000	3,420	„ 20	—	2,442	„ 12	251,300	66
„ 7	873,600	1,443	„ 25	—	183	„ 19	227,000	149
„ 9	960,000	42,840	„ 29	—	62	„ 26	767,600	53
„ 11	237,000	1,613	Oct. 2	167,327	2,122	April 2	625,650	236
„ 14	690,000	23,229	„ 4	90,090	—	„ 9	891,750	82
„ 16	270,000	25,433	„ 6	126,791	150	„ 16	3,963,000	74
„ 18	391,600	8,167	„ 9	313,200	—	„ 24	1,382,600	27
„ 21	1,125,000	722	„ 11	313,420	88	„ 30	624,650	29
„ 23	1,440,000	42,570	„ 13	196,560	—	May 7	1,431,150	32
„ 25	720,000	13,090	„ 16	698,443	817	„ 14	1,679,200	37
„ 28	630,000	10,467	„ 19	498,400	—	„ 21	511,500	249
Mar. 1	1,440,000	22,092	„ 20	859,600	—	„ 28	523,300	45
„ 3	1,440,000	5,761	„ 23	599,640	—	June 4	1,175,750	33
„ 6	984,000	130,200	„ 25	840,000	110	„ 11	1,151,050	26
„ 10	2,317,986	—	„ 27	590,604	—	„ 18	653,800	146
„ 15	3,058,760	—	Nov. 1	155,800	—	„ 26	2,387,400	590
„ 17	1,452,000	30,492	„ 3	—	136	July 2	449,200	—
„ 20	1,144,800	1,661	„ 6	473,040	—	„ 9	—	185
„ 22	891,660	4,697	„ 8	1,539,400	75	„ 16	703,100	26,680
„ 24	1,622,800	2,596	„ 10	270,650	—	„ 23	879,500	138
„ 27	912,825	9,006	„ 13	401,750	74	„ 30	403,200	351
„ 29	3,310,512	11,248	„ 16	150,400	—	Aug. 6	188,850	41
„ 31	2,516,832	—	„ 19	1,106,900	289	„ 12	365,900	—
April 3	1,217,100	1,056	„ 21	277,300	—	„ 19	236,100	1,890
„ 19	1,440,000	119	„ 23	213,800	60	„ 27	660,500	—
„ 25	—	211	„ 26	336,450	—	Sept. 2	699,050	81
„ 28	—	7,920	„ 28	1,762,950	3,799	„ 10	1,310,200	75
May 8	1,900,800	735	„ 30	562,050	—	„ 16	529,500	34
„ 10	—	217	Dec. 5	146,500	56	„ 24	—	337
„ 19	1,320,000	3,232	„ 7	138,600	—	Oct. 2	596,000	45
„ 22	—	4,422	„ 10	148,650	15	„ 10	799,800	4,650
„ 26	—	13,200	„ 12	937,300	—	„ 16	1,272,000	32
„ 29	—	60	„ 14	406,400	3,640	„ 25	1,108,800	9
„ 31	—	40	„ 17	140,000	—			

during the whole of the time the filter was working. Rain and snow were excluded from the tank by a covering of canvas towards the end of 1888, and this with the more even distribution of the sewage enabled, as stated above, nearly double the daily quantity of sewage to be treated.

For a month previous to the application of sewage to this tank it was, as already mentioned, filtering city water at a rapid rate. The number of bacteria in a c.c. of this water (obtained from the Merrimack river) was seventy-six, and in the effluent twenty-four. After applying sewage which contains from half a million to one million and a half bacteria per c.c., the number in the effluent increased rapidly, till at the end of ten days it was 387,000 per c.c., which in two weeks fell to 1,443. The same general condition of large numbers of bacteria coming through on the first daily application of sewage was found to be the case in all the tanks, irrespective of the filtering material or the protection or exposure of the tanks. The number in the effluent was found to vary from hour to hour, and to depend upon the rate of flow. Thus in December, 1888, the number of bacteria in the morning before sewage was applied was found to be forty-six; after sewage had been on an hour, and the flow at the outlet had increased twelve times, the number per c.c. was 1,230. The next sample contained 1,748, and when at the maximum rate of flow—*i.e.* 50 per cent. greater than the last—there were found 1,640 per c.c.; after this the number fell off in seven hours to about 200. On another occasion, in the morning the number was ninety-eight; a little after the maximum rate of flow the number was 16,478; two hours later it was 5,000; and after five more hours it was 1,300. This circumstance will help to explain

the great difference in the numbers of bacteria found in the effluent from day to day (see Table).

Experiments were made to determine whether the increased number of bacteria with the greater flow was due to an increased number coming through the sand of the filter at the time, or was due to washing out, in consequence of the greater flow, numbers that had accumulated or grown in the under-drains or gravel on the bottom exposed to air entering the outlet pipe. For this purpose the outlet pipe was closed, when water was slowly coming through the sand, for several hours, and the accumulated water drawn out at varying velocities. It was found that when this accumulated water was drawn at a slow rate very few bacteria were in it, and that the number increased greatly when drawn at a rapid rate, showing that some bacteria were then washed off from their resting-places in the drains and in the gravel; but this increase in the number of bacteria was not as great as that which was observed when the effluent was coming directly, at the greater rate, through the sand. Numerous other experiments were made, which proved conclusively that the great increase in the number with the increase in rate was due to more bacteria actually passing through the sand with the increased rate of flow.

The sand of this tank was examined for bacteria by boring a hole from top to bottom and taking out samples at different depths and determining the number of bacteria in 1 gramme of the sand derived from different depths. It is possible that in obtaining the specimen from the lower part of the tank some uncertainty may exist in the results from sand falling from the upper layers. The following table gives the results of these observations :—

Number of Bacteria found in 1 *grm. of Sand taken at Various Depths of Tank No.* 1 *on Different Dates*

Distance from surface	Dec. 19, 1888	Feb. 4, 1889	May 22, 1889
0 to ¼ inch .	132,400	262,800	—
0 to ½ „ .	—	—	1,760,000
½ to ¾ „ .	—	106,300	105,000
1¼ to 1½ „ .	—	—	207,200
2 inches . .	—	46,200	60,200
3 „ . .	43,600	—	111,300
4 „ . .	—	40,000	—
5 „ . .	—	—	63,400
8 „ . .	17,900	—	30,700
9 „ . .	—	14,600	—
12 „ . .	—	—	34,100
14 „ . .	—	8,900	—
15 „ . .	10,700	—	—
19 „ . .	—	—	12,300
24 „ . .	12,900	—	—
32 „ . .	—	6,900	31,300
36 „ . .	10,500	—	—
46 „ . .	—	—	4,900
48 „ . .	61,000 ?	6,200	—
60 „ . .	16,700	—	4,100

In general there is, therefore, a very marked decrease in number from the top to the bottom, there being in the bottom inch but 3 per cent. of the number found in the top inch ; but the decrease is most rapid in the first few inches at the top. These results were confirmed by several similar investigations made on different sand filters, and are in accordance with Piefke's examinations of the bacterial contents of the sand at different depths in waterworks filters (see p. 158).

The number of experimental tanks was upwards of twenty, and the investigations on each of these included innumerable chemical analyses of the raw sewage and of the effluent, besides the bacteriological examination of a very large number of samples.

In summarising the results obtained by the intermittent filtration of sewage through very coarse sand, as well as through sand which was practically fine

granular dust, through peat and through soil, the following may be taken as the percentage reductions effected in the number of bacteria present :—

Very coarse sand, 5 feet in depth (grains averaging about 0·06 inch in diameter), allowed from 26 to 40 per cent. of the bacteria to pass through.

Finer sand, 5 feet in depth (grains averaging about 0·006 inch in diameter), 14 per cent.

Very fine sand, 5 feet in depth (river silt), 5 per cent.

A mixture of coarse and fine sand and fine gravel, 3 feet 8 inches in depth, 5 per cent.

Ditto, plus an upper layer of 10 inches of yellow, sandy loam, and 6 inches brown soil, 5 per cent.

Garden soil, 5 feet in depth. So very few organisms were discoverable in the effluent that it is presumed that those found did not come through the filter, but were due to post-filtration sources.

Peat, 5 feet in depth. The latter remarks apply equally to this filter.

In the case of the two last-named filters, however, the rate of filtration was so slow as to render them entirely useless for practical filtration purposes.

Purification of sewage by irrigation.—In connection with the above results it will be interesting to learn what effect is produced on the bacterial contents of sewage by its treatment in sewage farms. Miquel[1] has examined the sewage of Paris before and after it has been led on to the land at Gennevilliers, and he finds that, whereas the raw untreated sewage contains on an average about 13,800,000 microbes per c.c., after passing through the soil it contains on an average about 7,475. The effluent from this land is returned to the

[1] '*Manuel pratique d'Analyse bactériologique des Eaux.*' Miquel. Paris, 1891, p. 139.

Seine in four drains, and the following table gives the results obtained :—

Average Bacterial contents of effluent from Gennevilliers
(Miquel, 1891)

	Number of Bacteria per c.c.
Drain of Asnières	410
„ „ Argenteuil	6,745
„ „ la Garenne. . . .	7,945
„ „ Epinay	14,795
Average	7,475

Miquel states that at the point where these drains communicate with the Seine the number of bacteria in the river rises to above 200,000 per c.c.

In connection with these results, however, we would point out that, in conducting investigations (whether bacteriological or chemical) on the efficiency of sewage purification on the large scale, it is necessary that the greatest caution should be exercised, otherwise the most fallacious results may be arrived at. Thus the composition of the raw sewage is continually varying, and is utterly different at different times of the day; it is, therefore, very difficult, and in some cases quite impossible, to institute comparisons between samples of raw sewage and effluent, whilst the difficulty is frequently further enhanced, especially in the case of sewage-works where land is employed, by the accession during purification of more or less spring-water to the sewage under treatment. It has long been customary in the chemical investigation of sewage-purification to look upon the chlorine as a rough indication of whether the raw sewage and effluent are comparable, and there can be no doubt that no reliance whatsoever should be placed on results in which the chlorine in the effluent differs materially from that in the raw sewage. The experiments of the Massachusetts Board of Health, referred to above, clearly indicate the enormous variation in the

number of bacteria in the filtered sewage at different periods of the flow, results which are in complete harmony with observations which have been made by one of us during the purification of sewage on the large scale in various places.

Experiments on a small scale with various filtering materials.—Although sand is the material almost invariably employed for the filtration of water on the large scale, yet we know that in domestic filters other substances are extensively used; and it was with a view of ascertaining the efficiency of different substances as regards their retention of microbes that the following series of experiments were conducted by one of us.[1]

The materials investigated were ferruginous and very finely grained greensand, silver sand, animal-charcoal, iron sponge, brick-dust, coke, vegetable and animal charcoal, and powdered glass; they were all used in a very fine state of division, the filters, which were all 6 inches in depth, being composed of particles which had passed through a sieve of forty meshes to the linear inch. In all cases the materials were sterilised before use. The results are embodied in the table on following page.

It will be seen that vegetable-carbon, whether in the form of charcoal or of coke, offers a very strong barrier to the passage of microbes. This material has been generally regarded as of but little value for water purification, owing, as in the case of sand, to its chemical inactivity; but as biological filters these substances occupy a high place, and owing to their cheapness should prove of great service in the purification of

[1] 'The Removal of Micro-organisms from Water.' Percy Frankland, *Roy. Soc. Proc.*, 1885. Also *Proceedings Institution of Civil Engineers*, 1886.

Efficiency of different Filtering Materials (Percy Frankland, 1885)

Filtering Material	Efficiency	Micro-organisms per c.c.		Reduction per cent.	Approximate rate of Filtration per square foot per hour
		Unfiltered Water	Filtered Water		
Ferruginous greensand (from Red Hill, Surrey)	Initial	80	0	100·0	—
	After 13 days' action	8,000	1,000	88·0	0·73 gallons
	After 1 month's action	1,280	780	39·0	1·14 ,,
Silver sand	Initial	11,232	1,012	—	More than 30 ins. per hr.
Powdered glass	Initial	11,232	792	—	More than 8 ins. per hr.
Brick-dust (pulverised red-brick)	Initial	3,000	730	76·0	—
	After 5 weeks' action	6,000	400	93·0	0·48 gallons
Iron sponge	Initial	80	0	100·0	—
	After 12 days' action	2,800	0	100·0	0·40 gallons
	After 1 month's action	1,280	2	99·8	0·45 ,,
Animal charcoal	Initial	Too numerous to count	0	100·0	—
	After 12 days' action	2,800	0	100·0	0·46 gallons
	After 1 month's action	1,280	7,000	Increase 447·0	0·86 ,,
Vegetable charcoal	Initial (2nd day)	9,700	0	100·0	—
	Initial (2nd day	2,898	0	100·0	0·22 gallons
	After 1 month's action	2,230	107	95·0	0·22 ,,
Coke	Initial	3,000	0	100·0	—
,,	After 5 weeks' action	6,000	90	98·5	0·50 gallons
,, Filter (*a*)	Initial (2nd day)	26,000	0	100·0	0·89 ,,
,, ,,	After 3 weeks' action	2,230	339	85·0	1·32 ,,
,, Filter (*b*)	Initial (2nd day)	26,000	0	100·0	0·67 ,,
,, ,,	After 3 weeks' action	2,230	219	90·0	1·03 ,,

N.B.—The filtering stratum was constructed exactly 6 ins. in depth, and the filtering material was with a few exceptions made to pass through a sieve of 40 meshes to the linear inch.

water. These materials, coke and vegetable charcoal, are also especially well fitted for use in breweries and distilleries, where it is so necessary to have a water which, though perfectly free from organic life, is at the same time free from antiseptic substances, such as iron, which militate against fermentation.

Here also on the small scale we have the importance of the rate of filtration emphasised, the greater rate which prevailed in the second series of experiments causing the efficiency to deteriorate more rapidly.

In the case of animal charcoal it will be seen that after one month's action there was a large increase in the numbers of microbes present in the filtrate, thus clearly demonstrating the necessity of its frequent renewal when used for filtration purposes.

We have appended the following table giving the chemical analysis of the water before and after passing

Chemical Analysis of Water before and after Filtration, through Coke and Charcoal respectively (Percy Frankland)

Results of Analysis expressed in parts per 100,000

—	Unfiltered Water	Water from Fine Coke		Water from Fine Wood Charcoal	Water from Coarse and Fine Wood Charcoal
		A	B		
Total solids . .	24·80	24·60	25·00	24·68	24·64
Organic carbon .	0·144	0·118	0·107	0·090	0·098
„ nitrogen .	0·050	0·040	0·038	0·024	0·031
Ammonia . .	0	0	0	0	0
Nitrogen as nitrates and nitrites .	0·190	0·209	0·202	0·221	0·217
Total combined nitrogen . .	0·240	0·249	0·240	0·245	0·248
Chlorine . .	1·9	1·9	1·9	1·9	1·9
Temporary hardness . . .	11·3	11·3	11·3	12·5	12·3
Permanent hardness	5·6	5·6	5·6	4·6	4·6
Total . . .	16·9	16·9	16·9	17·1	16·9

through these various filters, as showing again how insignificant may be the chemical action of materials causing a marked biological improvement; the samples were collected when the filters were working with their greatest efficiency.

Domestic Filters

Pasteur-Chamberland Filter.—In this filter the water is made to pass through a cylinder of biscuit-porcelain, the number of such cylinders depending of course upon the amount of filtered water required. The one experimented upon by one of us [1] consisted of ten such cylinders. Ordinary filtered Thames water was forced through under a pressure of 30 to 40 feet of water. Under these circumstances the filter commenced by yielding 1 litre in 40 minutes, or 36 litres per 24 hours; but already, at the end of a fortnight's continuous action, it was only delivering 1 litre in 1 hour 14 minutes, or rather less than 20 litres per 24 hours, and after $2\frac{1}{2}$ months the rate of filtration was 1 litre in 1 hour 22 minutes, or $17\frac{1}{2}$ litres in 24 hours.

Chemically again this filter was found to have but a very trifling influence upon the composition of the water, the only change being a slight diminution in the amount of mineral matter present. Biologically, on the other hand, it was, at any rate when new, very efficient, the complete removal of the micro-organisms being accomplished. Numerous and elaborate investigations as to the efficacy of this filter have since been made by various bacteriologists, with the result that, although in the first instance it is found to yield germ-free water, it loses this power after being in action a

[1] 'Removal of Micro-organisms from Water.' Percy Frankland, *Roy. Soc. Proc.*, 1885.

short time. Freudenreich [1] points out that although opinion in Germany is much divided as to the value of this filter, yet in France there appears to be no hesitation in accepting it as a thoroughly reliable purifying agent. Miquel [2] in commenting upon the results of some very carefully conducted experiments says: 'Par conséquent le filtre en biscuit de Chamberland est capable de retenir tous les organismes contenus dans les liquides.'

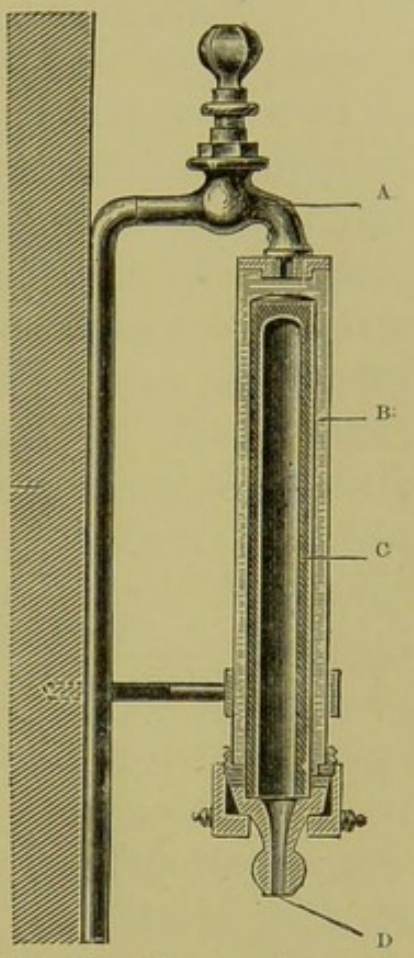


Fig 15. Pasteur-Chamberland Filter.

A, tap on service-pipe; B, external metal cylinder; C, porous porcelain cylinder; D, glazed porcelain delivery-tube for filtered water.

It is on account of the conflicting opinions expressed concerning this filter that Freudenreich [3] has made a special study of its behaviour as regards micro-organisms. He draws attention to the fact that the pressure under which the filter works is not able to force the bacteria through the pores of the biscuit-porcelain, and that the presence of organisms in the filtrate is due rather to their growth and multiplication within the pores of the filter, which takes place the more rapidly the higher the temperature of the room in which the filter is kept (Kübler, also Nordtmeyer, *Zeitschrift für Hygiene*, vol. x.). To satisfy himself as to this point, Freudenreich made the following series of examinations:—

[1] 'Ueber die Durchlässigkeit der Chamberland'schen Filter für Bakterien,' *Centralblatt für Bakteriologie*, vol. xii. 1892, p. 240.

[2] *Analyse Micrographique des Eaux.* Paris, 1891, p. 174.

[3] *Loc. cit.*

Bacterial Condition of Water filtered through a Chamberland filter and kept at 35° and 22° C. respectively (Freudenreich)

Filters kept at a Temperature of 35° C.

Examination 1, after	2	days	the water	was	sterile
2	„ 4	„	„	„	
3	„ 4	„	„	„	
4	„ 5	„	„	„	
5	„ 5	„	„	„	
6	„ 6	„	„	„	
7	„ 6	„	„	was	not sterile
8	„ 6	„	„		„
9	„ 7	„	„		„
10	„ 8	„	„		„
11	„ 10	„	„		„
12	„ 11	„	„		„
13	„ 11	„	„		„
14	„ 14	„	„		„
15	„ one month		„		„

Filters kept at a Temperature of 22° C.

Examination 1 after	7	days	the water	was	sterile
2	„ 8	„	„	„	„
3	„ 9	„	„	„	„
4	„ 10	„	„	„	„
5	„ 10	„	„	„	not sterile
6	„ 10	„	„	„	sterile
7	„ 11	„	„	„	not sterile
8	„ 11	„	„	„	sterile [1]
9	„ 11	„	„	„	not sterile [1]
10	„ 12	„	„	„	„
11	„ 12	„	„	„	sterile
12	„ 12	„	„	„	„
13	„ 15	„	„	„	„
14	„ 15	„	„	„	not sterile [1]
15	„ 18	„	„	„	sterile [1]

A filter kept at a temperature of between 15° and 18° C. yielded a sterile filtrate even after twenty-one days. From these experiments it would appear that certain bacteria are capable of growing through the filter, and others not; for whereas in some instances

[1] Freudenreich explains these discrepancies as due partly to differences in the character of the cylinders employed, and partly to differences in the micro-organisms present in the water at different times.

recorded in the above table the filtrate was quite sterile, an examination of another cylinder (of the same age, and working under similar circumstances) yielded a non-sterile liquid. Again, that when the filter is kept at a low temperature, the filtrate remains sterile over a much longer period of time, the conditions not being so favourable for the development and multiplication of the micro-organisms arrested in the pores of the filter. A coating of slime forms on the porcelain, often reaching a thickness of 1 mm. and more; this tends to retard the rate of filtration which we have seen takes place after the filter has been in use for a short time, and necessitates the cylinders being frequently thoroughly washed and sterilised; in fact, Freudenreich, in recommending these filters, states that they should not be kept in use for longer than eight days without being sterilised, care being at the same time taken that the temperature of the place where they are kept is as low as possible.

These results raise, of course, the important question as to whether pathogenic organisms, such as cholera and typhoid bacilli, are able to grow through the pores of such a filter, and Freudenreich has also conducted some experiments with a view to determining this point. For this purpose a Chamberland porcelain cylinder was immersed in sterile broth, which latter of course penetrated also into its interior; typhoid bacilli were then introduced into the outer broth, in which they abundantly multiplied, but the internal broth remained sterile even after twenty-two days, although the apparatus was maintained throughout at the temperature of 35° C. It would thus appear that the typhoid bacilli were not able to grow through the pores of the porcelain; but the result of the experiment bears, as Freudenreich points out, another in-

terpretation also, for the internal sterile broth, on examination, was found to be incapable of supporting the growth of typhoid bacilli, doubtless because the products of the growth of the typhoid bacilli in the external broth had diffused through the porcelain into the internal broth, unfitting the latter for the nutriment of typhoid bacilli; for, as is well known, this bacillus will not develop in media which have already supported growths of typhoid bacilli. It must be regarded, therefore, still as an open question whether pathogenic organisms, such as typhoid bacilli, can or cannot grow through the pores of the Chamberland filter; and until this question has been answered in the negative it is obvious that in using these filters the cylinders should be frequently cleaned and sterilised, which is, in fact, also necessary on the ground of the rapid reduction in the yield of water which takes place.

Berkefeld Filter.—One of the most important of the modifications of the Chamberland filter which has been introduced is the Berkefeld filter.[1] It is constructed upon exactly the same principles as the Chamberland filter, but instead of biscuit-porcelain baked infusorial earth (Kieselguhr) is used. It yields, in the first instance, sterile water, and can be easily cleaned and re-sterilised, when necessary, by placing it in a vessel containing cold water, and allowing it to boil for three-quarters of an hour. The material being somewhat brittle, care must be used in handling it, and for the same reason, for cleansing purposes, it should not be placed, to begin with, in boiling water, but gradually heated as described. It may also be cleansed by rubbing the cylinder with a loofah under running water;

[1] 'Ueber Wasserfiltration durch Filter aus gebrannter Infusorienerde,' Nordtmeyer. *Zeitschrift für Hygiene*, vol. x. p. 145, 1891; also *loc. cit.* p. 155, a paper by Bitter on the same filter.

in this manner all impurities which may have collected on the surface are easily and quickly removed (Bitter).

The Berkefeld cylinders, of 26 cm. length, 5 cm. external diameter, and 1 cm. thickness, yield under a pressure of $3\frac{1}{2}$ atmospheres ·75 litre per minute when constructed of the closest texture, 2 litres when of medium, and 3·45 litres per minute when of the most porous make. Hence it has in respect of rate of filtration a decided advantage over the Chamberland filter, whilst as regards the removal of bacteria it is equally efficacious.

Nordtmeyer's experiments on this Berkefeld filter show, as already mentioned in the case of the Chamberland filter, that the presence of bacteria in the filtrate is independent of the numbers in the unfiltered water, but due rather to the growth and multiplication of the micro-organisms through the filter-pores, which organisms, becoming detached, get washed into the filtrate. Thus he found that when large quantities of microbes appeared in the filtrate, this only occurred in the first quantity drawn off, and that on allowing a few litres to run to waste the filtrate became again nearly sterile. The experiments of Freudenreich on the Chamberland filter amply confirm these results obtained by Nordtmeyer on the Berkefeld.

Kirchner[1] has recently examined this filter as regards its retention of cholera and typhoid bacilli. The experiments were conducted in the following manner:— 100 c.c. of a fresh broth-culture of cholera or typhoid bacilli were added to every litre of river Ihme water employed (a small stream in the vicinity of Hanover,

[1] 'Ueber die Brauchbarkeit der Berkefeld-Filter,' *Zeitschrift für Hygiene*, vol. xiv. p. 307, 1893. See also *ibid.* vol. xv. p. 179, 1893; also 'Gesichtspunkte für die Prüfung und Beurteilung von Wasserfiltern,' Gruber. *Centralblatt für Bakteriologie*, vol. xiv. p. 488, 1893.

and containing about 30,000 organisms in 1 c.c.). The filter was first sterilised and then fed with this infected water, an unfiltered drop of which was examined at the commencement of the experiment, and filtered drops at the end of twenty-four hours. The cholera bacilli were found in the unfiltered water on the first and second day, but at the end of forty-eight hours, when it had become very putrid, none were detected. They were present at the end of twenty-four hours in the filtered water, and were still found at the end of thirty-five hours, but none were discoverable at the end of forty-eight hours. The typhoid bacilli were found in the unfiltered water in large numbers at the end of seventy-three hours, when the investigation was discontinued; in the filtrate they did not appear until after forty-eight hours, and were still present in large numbers at the end of seventy-three hours. It should be pointed out that in consequence of the large admixture of broth to the water under examination the latter must have been very highly charged with nutritive material favouring the growth and multiplication of the bacteria present, and therefore the conditions are different from those under which natural waters actually containing typhoid and cholera germs might be subjected to filtration. Also as regards the presence of typhoid bacilli in the filtrate, it is to be regretted that Kirchner gives no particulars as to the manner in which these were identified, a very serious omission, considering the ease with which a wrong diagnosis of their presence may be made. (For the important services rendered by the Chamberland and Berkefeld filters in the laboratory, see p. 4.)

Schöfer [1] in examining the Berkefeld filter with the

[1] 'Ueber das Verhalten von pathogenen Keimen in Kleinfiltern.' *Centralblatt für Bakteriologie*, vol. xiv. p. 685, 1893.

special object of ascertaining its power of retaining typhoid bacilli, purposely introduced with the latter as small a quantity as possible of nutritive material into the sterilised water which was to be filtered, thus reducing the chances of multiplication and subsequent growth of the organisms through the pores of the filter. Observing this precaution, a filter fed with sterilised distilled water infected with typhoid bacilli yielded, when preserved at a temperature of from 19–26° C, a sterile filtrate for a period of sixteen days, similar results being also obtained when sterilised tap water containing typhoid organisms was used.

In another experiment, 7 litres of a well-water rich both in bacteria (2,960 per c.c.) and organic matter was first passed through the cylinder, in order to obtain a deposit of organic material and so possibly to favour the growth of the typhoid bacilli; the cylinder was then sterilised and typhoid-infected water passed through, but although during the twenty-four days over which the investigation extended fresh typhoid bacilli were introduced no less than twelve times, none were discoverable in the filtrate. In this, as in the previous experiments, there was obviously not sufficient food material present to permit of the growth and multiplication of the typhoid bacilli. A further experiment was made using very impure canal water (182,000 bacteria in 1 c.c.), receiving indeed a large part of the raw sewage of Vienna; 4 litres of this were passed through a Berkefeld cylinder to coat it with an organic deposit. The cylinder was then sterilised and filtration continued from July 3–15, 1893, using the same canal water to which on July 3, 6, 9, 11, and 14, typhoid bacilli were added, the filtrate remaining however quite sterile until after the addition of bouillon (5 c.c. per 600 c.c. water) on July 15, which addition was followed

on July 17 by the appearance of typhoid bacilli in the filtrate. This result clearly indicates that *even the polluted canal-water did not in itself contain the necessary pabulum for the typhoid bacilli to grow through the filter-pores*, and that this was only rendered possible after the addition of the bouillon.

From another filter [1] fed with typhoid germs, the filtrate was sterile to start with; on the following day 5 c.c. of sterile bouillon were added, and two days later typhoid bacilli appeared in the filtrate; but they gradually decreased in numbers, although fresh typhoid organisms were twice introduced into the unfiltered water. After an interval of seven days 5 c.c. of bouillon were again added to the water, and the number of typhoid organisms in the filtrate rose on the following day from 9 to 6,139 per c.c. without any fresh infection having been made. The large increase was due to the rapid multiplication of the few isolated typhoid bacilli remaining in the pores of the filter, in consequence of the supply of food material in the shape of bouillon.

Schöfer is of opinion that typhoid bacilli, as ordinarily present in water, are not supplied with the requisite conditions for their growth and multiplication, and are, therefore, incapable of growing through these filters and reaching the filtrate; these conditions are, however, furnished when a sufficient supply of food material is contained in or added to the liquid to be filtered, in which case the cylinders are no longer able to retain the typhoid bacilli. These experiments explain the unsatisfactory results obtained by Kirchner in his investigations referred to above, and at the same time indicate the nature of the precautions which should

[1] The pure water (Hochquellwasser) of the Vienna supply was used, and no previous deposit of organic material was secured.

be adopted in testing the bacterial efficiency of such filters.

Stone Filters.—These filters have for a long time been regarded with favour, because they succeed in producing a liquid bright and clear to the eye, removing from visibly turbid water the coarser particles in suspension. Some experiments as to their bacterial efficiency have recently been made by Esmarch.[1] He investigated six stone filters coming from such different parts of the world as the Canary Islands and Mexico. In all cases he found them absolutely unreliable, large quantities of bacteria being found in the filtrate.

Asbestos Filters.—Elaborate experiments have been made by Gruber-Weichselbaum,[2] and later by Jolles,[3] on the value of asbestos as a filtering material. The result of these investigations shows that, although in the first instance filters in which this material is used may be regarded as satisfactory, they do not long continue to retain the micro-organisms. The passage of the bacteria is attributed by these investigators to irregularity in the pressure of the water supplied, which gradually leads to the disruption of the layer of asbestos, and by creating even the smallest rents facilitates the penetration of the micro-organisms into the filtrate.

STERILISATION OF WATER BY HEAT

At the present time, when such grave suspicion has fallen upon filters as a class, it is becoming not an uncommon practice with many to boil suspicious water

[1] 'Ueber Wasserfiltration durch Steinfilter,' *Centralblatt für Bakteriologie*, 1892, vol. xi. p. 525.

[2] 'Ueber die Wirksamkeit von Asbestfiltern zur Gewinnung von sterilem Wasser,' *Oesterr. Sanitätswesen*, 1891, No. 43.

[3] 'Untersuchung über die Filtrationsfähigkeit des patentirten Wasserfilters Puritas,' *Centralblatt für Bakteriologie*, vol. xii. p. 596, 1892.

intended for drinking, and thus to dispense entirely with filters, or, at most, to use them only for aërating the water after boiling, and so remove the flat and vapid taste possessed by boiled water. It will be of importance, therefore, to enquire as to what is the effect of high temperatures upon bacteria in water. Miquel[1] gives the following tables, which show that it is between 14° C. and 50° C. that the majority of the micro-organisms are destroyed. But this diminution must necessarily depend upon the nature of the microbes present in the water, micrococci being far more readily destroyed than bacilli, whilst some bacilli, again, are far more resistant to high temperatures than others. Miquel sums up his results with the following reassuring remark :—'De cet ensemble de faits, on peut conclure qu'en l'absence de filtres parfaits, on débarrasse les eaux suspectes de presque tous les microbes qu'elles renferment en les chauffant durant quelques minutes. L'expérience ayant démontré, d'autre part, qu'il n'existe pas de bactéries pathogènes dont les germes puissent résister à 100°, on peut conclure que l'ébullition préalable des eaux est un moyen prophylactique certain contre les épidémies qui ont pour origine la diffusion des bactéries par les eaux potables.'

Bacterial Condition of different Waters when heated to various Temperatures during 15 *minutes* (Miquel)

Seine Water

Temperature maintained	Number of Microbes in 1 c.c.
20° C.	464
45° C. during 15 minutes	396
55° C. „ „	33
65° C. „ „	20·8
75° C. „ „	9·6
85° C. „ „	6·6
95° C. „ „	2·8
100° C. „ „	3·3

[1] *Analyse micrographique des Eaux*, Paris, 1891, p. 180.

Seine Water

Temperature maintained	Number of Microbes in 1 c.c.
22° C.	848
43° C. during 15 minutes	640
50° C. „ „	132
60° C. „ „	40
70° C. „ „	27·2
80° C. „ „	26·4
90° C. „ „	14·4
100° C. „ „	5·2

River Ourcq Water

Temperature maintained	Number of Microbes in 1 c.c.
14° C.	460,800
50° C. during 10 minutes	600
60° C. „ „	(60)
70° C. „ „	88·8
80° C. „ „	62·4
90° C. „ „	26·4
100° C. „ „	0·5
100° C. during 20 „	0·0

Vanne Water

Temperature maintained	Number of Microbes in 1 c.c.
13° C.	4,800
50° C. during 10 minutes	175
60° C. „ „	—
70° C. „ „	3
80° C. „ „	1·7
90° C. „ „	0·3
100° C. „ „	0
100° C. during 20 „	0

We would refer the reader to the chapter on the multiplication of micro-organisms, where an account is given (p. 229) of experiments on the capacity of boiled water to subsequently support bacterial life.

As regards the effect of high temperatures on pathogenic organisms purposely introduced into water, the experiments of Currier [1] throw some light. The organisms were introduced into unsterilised spring water

[1] 'Sterilization of Water,' *New York Medical Record*, No. 1023, 1890, p. 680.

or ordinary tap water, and plate-cultivated after exposure to different degrees of heat for varying periods of time. It was found that a temperature of 100° C., maintained during ten minutes, was required to destroy the spores (?) of the tubercle bacilli, whilst the spores of anthrax were killed off in five minutes. Other pathogenic microbes are even more sensitive to high temperatures. Pyogenic micrococci, the typhoid and diphtheria bacilli, as well as the microbes of malaria [1] were destroyed when the water was heated to boiling and then allowed to cool, whilst the Comma bacilli in water could not survive even a momentary exposure to 70° C.

For the complete sterilisation of water by heat Currier states that a temperature of 100° C. maintained for fifteen minutes, except in the case of most extraordinarily resistant microbes, is sufficient, whilst boiling for five minutes already eliminates the risk of using water containing those pathogenic forms, like the cholera and typhoid germs, which are usually regarded as a source of danger in water.

THE PURIFICATION OF WATER BY MEANS OF SEDIMENTATION

In the practice of water-engineering there is no method of improving the quality of surface-waters which has been so much taken advantage of as that of causing them to remain at rest for a longer or shorter period of time in large reservoirs. In this manner waters which are turbid and unpleasing to the eye not only become comparatively clear and bright by the subsidence of mechanically suspended particles, but if the storage be sufficiently prolonged, a very considerable reduction in the amount of dissolved organic matter may also take

[1] These were the bacilli at that time believed by some to be the inducing cause of malaria.

place. Such improvement is, in fact, most conspicuous in the case of the great natural storage reservoirs constituted by lakes.

This principle of subsidence has not only been employed in the improvement of water intended for domestic and manufacturing purposes, but it has also been abundantly resorted to for the purification of sewage and other refuse liquids.

Moreover, recognising the observed fact that the improvement in water during subsidence is the more rapid and pronounced the greater the amount of suspended matter initially present, the method of purification by subsidence has been developed by the artificial production of large quantities of suspended matter by the addition of precipitants. Such purification by precipitation has been principally attempted in the case of sewage, but it has also been employed in the shape of the well-known Clark's process for the treatment of hard waters, in which, as has been long ascertained, there is not only a removal of the temporary hardness, but also a marked reduction in the amount of dissolved organic matter.

With the development of the new methods of bacteriological water examination, it became obviously a matter of primary importance, therefore, to investigate what was the bacteriological value of these several methods of water purification which had been so long in practical use by water and sewage engineers, and the chemical value of which had already been accurately determined.

These considerations led one of us, as early as 1884, to carry out laboratory experiments on the bacteriological value of these subsidence processes in their more important modifications, which investigations were conducted concurrently with those already de-

scribed on the bacteriological efficiency of different filtering media (see p. 169). The results of these investigations will be detailed later on in the present chapter (see p. 193), whilst in the first instance we will consider the subsidence phenomena which take place in natural lakes and in large reservoirs.

We have already had occasion to refer to the diminution in the number of micro-organisms which takes place in the water of the river Spree after it enters the Havel lake, and it was pointed out that, although the bacterial improvement in the water was in part due to the admixture of a certain quantity of pure spring water, yet that in the main this diminution in the number of micro-organisms must be ascribed to the process of sedimentation which there takes place. The more sluggish movement of the water in the wide expanse of the lake must facilitate the attachment of the microbes to particles of both mineral and organic matter, whilst a further opportunity is afforded them of clinging together amongst themselves, and forming zoogloœa masses, both of which circumstances cause their removal from the general body of water, and lead to their progress in a downward direction. We may also refer to some incidental observations bearing a similar explanation by Tils in his paper on the Freiburg water, already noticed (see p. 99), in which it is pointed out that the number of micro-organisms was invariably smaller in the water collected from the reservoir than in that taken from the source supplying the latter. This water being very pure, Tils ascribes the diminution in micro-organisms in the reservoir to the limited amount of food-material available, rendering them incapable of multiplying, and causing a certain number of them to die off. We are inclined, however, to attribute this decrease in the reservoir to the process of

sedimentation, which the following experiments,[1] quite recently carried out by one of us, demonstrate as taking place under such circumstances. Samples of water were taken from the River Thames at Hampton, where the intake of the Grand Junction Company is situated; secondly, from the first small storage reservoir nearest the river; thirdly, from the second small storage reservoir further from the river; and fourthly, from the large storage reservoir, the greater part of the water in which had been stored for six months, and none for less than one month.

The following results were obtained:—

	Number of Colonies obtained from 1 c.c. of water
1. Intake from Thames, June 25, 1892	1,991
2. First small storage reservoir	1,703
3. Second small storage reservoir	1,156
4. Large storage reservoir, (*a*) leeward side	464
4. „ „ (*b*) windward side	368

These figures bring out most strikingly the diminution in the number of suspended bacteria which is effected by storage. In the small storage reservoirs referred to above, the capacity is so limited that the water has but little chance of depositing anything, and we find accordingly the number of micro-organisms to be practically identical with that in the river-water feeding them, although there is a slight reduction perceptible. On the other hand, in the large storage reservoir the prolonged subsidence has brought about a most remarkable diminution in the number. There was a strong wind on the day the experiments were made, the surface of the water being considerably disturbed. It was anticipated, therefore, that the surface water on the lee side would probably contain more micro-

[1] 'Reinigung des Wassers durch Sedimentirung,' Percy Frankland. *Centralbl. f. Bakter.* xiii. p. 122, 1893.

organisms than that on the windward side, which was sheltered by the bank, and on this account samples were collected on both sides, with the result recorded above, that the water on the sheltered or windward side did actually yield fewer colonies than that on the more disturbed or lee side of the reservoir. It should be pointed out, however, that the real reduction in the number of bacteria effected by this storage was undoubtedly very much greater than that which is indicated by the above figures, for the reservoir must have been filled with Thames water, containing, as our previous experience leads us to know, a much larger number of micro-organisms than was present in the river at the time [1] these experiments were made.

The second series of experiments on Thames water was made on October 3, 1892, at the works of the West Middlesex Company at Barnes. Here the Thames water, pumped at Hampton, is made to pass through one, and in some cases two, storage reservoirs before being conducted on to the filter beds. Samples were collected of the Thames water coming directly from Hampton; secondly, of the water after having passed through one storage reservoir only; and thirdly, of the water after having passed through two storage reservoirs.

The following results were obtained:—

	Number of Colonies obtained from 1 c.c. of water
1. Thames water from Hampton	1,437
2. Ditto after passing through one storage reservoir	318
3. Ditto after passing through two storage reservoirs	177

Another series of experiments on the effect of storage was carried out at the Stoke Newington works of the New River Company, which were visited on August 27,

[1] See Tables, pp. 121–123, showing the variations in the number of bacteria in Thames water at different seasons of the year.

1892. At these works the water of the river Lea, mixed with a certain proportion of well-water, is brought along an artificial cutting, and is made to pass through two large reservoirs before going on to the filter beds. Samples were taken at the cutting just above the reservoirs, at the outlet of the first reservoir, and at the outlet of the second reservoir.

The following results were obtained:—

	Number of Colonies obtained from 1 c.c. of water
1. Cutting above reservoir	677
2. Outlet of first reservoir	560
3. Outlet of second reservoir	183

The above figures show that by storage alone the waters of the Thames at Hampton and the purer water of the New River Company's channel may, so to speak, be brought to the same bacteriological level.

More recently still we have met with an example of bacterial subsidence in a semi-natural lake, the Loch of Lintrathen, from which the water supply of Dundee is now obtained, and in which the water of the loch itself was found to contain a strikingly smaller number of bacteria than the streams supplying it; for the particulars of this case see p. 112 in the chapter on the bacterial contents of various waters.

On the Continent we rarely hear of storage reservoirs being employed, but in America they are more common, and such reservoirs are said to be frequently used for the waters of the Mississippi and Missouri rivers.[1] The water is allowed to remain at rest for many hours in large shallow settling basins, after which it is drawn off through a wire screen to retain fish and other large objects swimming in the water, and the sediment removed at regular intervals from the basins. The sedi-

[1] *Potable Water*, Floyd Davis, Boston, 1891, p. 83.

ment in the Missouri river at St. Louis, at certain seasons of the year, amounts to 1·8 per cent. of the bulk of the water. About 94·5 per cent. of this sediment is deposited in the settling basins in twenty-four hours, during ordinary states of the river; but for two months in the spring of each year no convenient length of time for settling will clarify the water. That in thus endeavouring to remove the coarser suspended particles an immense bacterial improvement is brought about in these river waters there can be no doubt; unfortunately no bacteriological investigations have been made in this direction, but from the experiments made by one of us on the London reservoirs and elsewhere it is obvious that the reduction in the number of micro-organisms present must be very important. This factor of subsidence comes into play in more than one industrial process for the purification of water on the large scale.

Clark's Process.—The most important of these methods of water-purification is that well known as Clark's Process, in which the 'temporary hardness' or soluble bicarbonate of lime present in water is converted into insoluble normal carbonate of lime by the addition of a suitable proportion of lime-water, with the result that both the lime originally present as bicarbonate as well as that added in the form of lime-water are precipitated. The bacteriological effect of this process has been investigated by one of us, both on the laboratory scale (see p. 196) as well as in the form in which it is carried out at waterworks and for industrial purposes.

Thus, at the Colne Valley Water-works, Herts, the hard water derived from deep-wells sunk into the chalk is mixed with the requisite proportion of clear lime-water, and then allowed to settle in open tanks. The subsidence of the precipitated carbonate of lime is so

rapid that under favourable circumstances the upper layers of water are, after three hours' time, fit for distribution. Samples of the unsoftened and softened water were examined[1] with the following results :—

	Number of Micro-organisms in 1 c.c. of water
Unsoftened water	322
Water after softening and two days' subsidence drawn from the main service-pipe . .	4
Reduction in the number of micro-organisms = 99 per cent.	

Gaillet and Huet's Process.—In this ingenious modification of Clark's process the water under treatment is mixed with the requisite proportion of lime-water and caustic soda (this effects the precipitation of some of the lime present as sulphate or chloride), and the whole is then made to pass upwards in a sinuous channel through a tower provided with a series of oblique diaphragms, which latter accelerate the deposition of the carbonate of lime. The passage through this tower occupies a period of two hours, during which the organisms become entangled in the carbonate of lime precipitate, and are separated with the latter from the water, as is seen from the following examinations made by one of us of the water before and after treatment :—

	Number of organisms in 1 c.c. of water
Artesian Well at Clyde Wharf, London ; untreated water from tanks	182
Ditto after treatment by Gaillet and Huet's process	4
Reduction in the number of micro-organisms = 98 per cent.	

The great practical importance of these results is sufficiently obvious, demonstrating, as they do, how by means of Clark's process the most remarkable diminu-

[1] *Proceedings of Institution of Civil Engineers*, 1886, Percy Frankland, on 'Water Purification.' For a fuller description of these methods, see article by the same on 'Water,' in *Thorpe's Dictionary of Chemistry applied to the Arts, &c.*, 1893.

tion in the bacterial contents of water may be effected. Such treatment, carefully carried out, must, therefore, in the case of any water liable to dangerous contamination, afford the most substantial obstacle to zymotic poisons reaching the consumer.

Röckner-Rothe's Process.—This is a somewhat similar process for purifying sewage or foul waters of any kind.[1] It consists in collecting the sewage into an enclosed well and slowly (2–9 mm. per second) pumping it up into an iron cylinder. Whilst the water is slowly rising in this cylinder a process of sedimentation takes place in which the particles in suspension, including bacteria, sink slowly to the bottom; moreover layers of slime gradually coat the inside of the cylinder, which offer points of attraction and adhesion for micro-organisms subsequently introduced. The purified liquid is drawn off at the top of the cylinder by means of a lateral pipe. The importance of this formation of slime is so far recognised that in order to stimulate its production chemicals, according to the nature of the water under treatment, are mixed with the sewage before it is forced up the cylinder. According to the bacteriological investigations of Wahl in Essen, Blasius in Brunswick, and Kaysser in Dortmund, the results are highly satisfactory. Wahl[2] records the most remarkable result that, whereas in the water before treatment 1,686,000 and 5,245,000 micro-organisms were present in a c.c., the same water on leaving the cylinder had only between 34 and 178 in a c.c.

The principle of sedimentation enters also largely into all the various processes of sewage purification by chemical precipitation. For by far the most extensive

[1] *Centralblatt für Bakteriologie*, vol. ii. p. 202, 1887.

[2] 'Mittheilungen über bacteriologische Untersuchungen Essener Abwässer,' *Centralblatt f. allgem. Gesundheitspflege*, v.

series of investigations on this important subject we are indebted to the Massachusetts Board of Health. A detailed description of their experiments and of the results arrived at is given at the close of the present chapter (see p. 205).

We shall in the next instance pass on to a description of experiments made on the small scale with a view of ascertaining the effect of the subsidence of solid particles of different kinds in water containing bacteria in suspension. It was by means of these laboratory experiments in fact that the remarkable removal of micro-organisms by subsidence and entanglement in solid particles was first brought to light and demonstrated.

Purification of Water by Agitation with solid Particles and subsequent Subsidence

Experiments on a small scale.—The first experiments of this kind were made by one of us in 1885.[1] In these investigations water containing micro-organisms was shaken up for a definite length of time with a given quantity of finely-divided material (made to pass through a sieve of forty meshes to the linear inch and subsequently sterilised). The water was then allowed to subside, and the clarified liquid submitted to examination as soon as possible after complete subsidence had taken place, as it appeared probable that, if the organisms were simply carried to the bottom by the subsiding particles without suffering any injury, they would rapidly again become distributed through the upper layers of water by multiplication. This redistribution of the micro-organisms would of course to a certain extent depend upon the character of the particular varieties in the water, *e.g.* whether they were

[1] 'The Removal of Micro-organisms from Water,' Percy Frankland. *Proc. Roy. Soc.* 1885. *Proceedings Institution of Civil Engineers*, 1886.

motile or not, whilst their power of multiplication would also depend upon the particular forms present, and upon their vital activity. As a matter of fact, in some cases a subsequent redistribution of the organisms was found to take place, and in others not.

The following are some of the principal results obtained in these investigations :—

Agitation with spongy iron.—The water was shaken with one-tenth of its weight of this material for fifteen minutes. The water was allowed to subside for half-an-hour before examination.

Bacterial Purification of Water by means of Agitation and Subsidence (Percy Frankland, 1885)

Untreated water contained 609 micro-organisms in 1 c.c.
After 15 minutes' agitation with spongy iron and 30 minutes' subsequent subsidence . 63 „ „
Reduction in the number of micro-organisms = 90 per cent.

Again, in another experiment :

Untreated water contained 155 micro-organisms in 1 c.c.
After 15 minutes' agitation with spongy iron and 30 minutes' subsequent subsidence . 10 „ „
Reduction in the number of micro-organisms = 93 per cent.

Agitation with chalk.—Water was shaken for fifteen minutes with one-fiftieth of its weight of chalk, and then allowed to subside for five hours :—

Untreated water contained 8,000 micro-organisms in 1 c.c.
After 15 minutes' agitation with chalk and 5 hours' subsequent subsidence . . . 270 „ „
Reduction in the number of micro-organisms = 97 per cent.

Agitation with animal charcoal.—The same water as that used in the last experiment was shaken with one-fiftieth of its weight of animal-charcoal for fifteen

minutes, and then allowed to subside for nearly five hours :—

Untreated water contained 8,000 micro-organisms in 1 c.c.
After 15 minutes' agitation with animal charcoal and nearly 5 hours' subsequent subsidence 60 ,, ,,
Reduction in the number of micro-organisms = 99 per cent.

Agitation with vegetable charcoal.—Water containing soil-extract was shaken with one-fiftieth of its weight of ordinary wood-charcoal for fifteen minutes, and was then allowed to subside for twenty-seven hours :—

Untreated water . . 3,000 micro-organisms in 1 c.c.
After 15 minutes' agitation with wood-charcoal and 27 hours' subsequent subsidence . . 120 ,, ,,
Reduction in the number of micro-organisms = 96 per cent.

Agitation with coke.—Water to which a little stale urine had been added was shaken with one-fiftieth of its weight of fine coke for fifteen minutes, and then allowed to subside for forty-eight hours :—

Untreated water . . Micro-organisms in 1 c.c. too numerous to count.
After 15 minutes' agitation with coke and 48 hours' subsequent subsidence . None.
Reduction in the number of micro-organisms = 100 per cent.

Further experiments made with coke and water containing soil-extract illustrate what we have said as to the reascension and multiplication of micro-organisms in some cases. Thus :—

Untreated water (containing soil extract), 3,000 micro-organisms in 1 c.c.
After agitation with coke and 26 hours' subsidence . . . . 20,000 ,, ,,

When less time was allowed for subsidence, the results were more satisfactory. Thus :—

Untreated water (containing soil extract) 655 micro-organisms in 1 c.c.
After agitation with coke and 5 hours' subsidence . . . . . 28 ,, ,,
Reduction in the number of micro-organisms = 96 per cent.

Water was also agitated with several other substances, such as china-clay, brick-dust, plaster of Paris, oxide of manganese, &c.; all of these, however, yielded less satisfactory results.

Laboratory experiments with 'Clark's process.' [1]—For testing the efficiency of this process on the laboratory scale, three stoppered Winchester quart bottles were taken, and to each were added 2 litres of ordinary London (Thames) water, to which a convenient proportion of organisms had been imparted by the addition of a little urine water. To two of these bottles 100 cubic centimetres of clear lime-water were added, this being calculated to remove 11·6 parts of carbonate of lime per 100,000 parts of the water. Each of these bottles was violently shaken, and the contents were then allowed to subside for eighteen hours. The two bottles to which the lime-water had been added were tested without disturbing the precipitate, as was also the third bottle, which had been left at rest in the same place as the other two. These tests showed the following numbers of micro-organisms to be present in the water before and after treatment :—

Untreated water at the outset contained	85	organisms in 1 c.c.
Ditto after 18 hours' rest . .	1,922	„ „
Water after treatment by Clark's process and 18 hours' subsidence	42	„ „

Reduction in the number of micro-organisms present in the original water = 51 per cent.

In order to appreciate the value of the treatment by Dr. Clark's process, it is necessary that the treated waters should be compared not only with the original water, but also with the untreated water after eighteen hours' rest; for the latter obviously indicates what the

[1] *Proceedings of Institution of Civil Engineers*, 1886. Percy Frankland.

condition of the water would have been at the time of examination, if no lime-water had been added. It appeared probable that after the subsidence of the carbonate of lime precipitate had taken place, the organisms which had been carried down by the latter would again become distributed throughout the upper layers of the water. In order to ascertain whether this was the case or not, the same waters, which had remained stoppered up and at rest, were again examined after the lapse of ten days. It was then found that the untreated as well as the softened waters contained immense numbers of organisms in their upper layers. In another series of experiments carried out under the same conditions, excepting that twenty-one instead of eighteen hours were allowed for the subsidence of the carbonate of lime, a reduction in the number of organisms amounting to 41 per cent. was obtained.

More recently an extended series of experiments on the same subject has been made by Krüger ;[1] they are described in the *Zeitschrift für Hygiene* for 1889. In his experiments Krüger has made a practice of examining bacteriologically, not only the upper layers of the water before and after treatment, but also water taken from the middle and bottom of the vessel. These investigations confirm in a very striking manner the evidence previously obtained by one of us of the subsequent redistribution and multiplication of the micro-organisms taking place in some cases after subsidence.

The following is a tabulated account of the numerous experiments carried out by this author :—

[1] 'Die physikalische Einwirkung von Sinkstoffen auf die im Wasser befindlichen Mikroorganismen,' *Zeitschrift für Hygiene*, vol. vii. p. 86, 1889.

Purification of Water by means of Agitation with solid Particles (Krüger)

Pulverised and Sterilised Potter's Clay

Temperature at which vessels were kept = 7° C.

—	Control vessel			Experimental Vessel No. 1 [1]			Experimental Vessel No. 2 [1]		
Before treatment . .	5,400 Bact. in 1 c.c.			5,101 Bact. in 1 c.c.			4,940 Bact. in 1 c.c.		
Clay added . . .	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	5,340	6,110	5,480	575	887	33,495	365	677	43,630
„ „ 20 „	5,960	6,710	6,210	521	155	43,595	121	53	150,320
„ „ 50 „	7,230	5,987	6,924	6,933	6,190	66,350	3,944	4,184	171,460

[1] Water was only slightly turbid after 12 hours.

Pulverised and Sterilised Calcium Carbonate

Temperature at which vessels were kept varied between 8°–10° C.

—	Control vessel			Experimental Vessel No. 1 [1]			Experimental Vessel No. 2 [2]		
Before treatment . .	7,432 Bact. in 1 c.c.			6,108 Bact. in 1 c.c.			13,650 Bact. in 1 c.c.		
Calcium carbonate added . . .	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	6,940	8,440	6,220	628	1,590	9,480	1,050	1,460	275,600
„ „ 20 „	8,796	7,970	9,140	700	691	231,020	807	570	887,020
„ „ 50 „	11,735	10,825	12,354	23,185	10,650	81,070	5,325	4,090	346,680

[1] Very slightly turbid in the lower layers after 19 hours.
[2] Ditto after 26 hours.

Finely Sifted and Sterilised Infusorial Earth

Temperature at which vessels were kept varied between 6°–9° C.

—	Control vessel			Experimental Vessel No. 1 [1]			Experimental Vessel No. 2 [2]		
Before treatment . .	6,030 Bact. in 1 c.c.			6,450 Bact. in 1 c.c.			6,840 Bact. in 1 c.c.		
Infusorial earth added	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	6,170	6,648	6,510	584	805	25,332	939	572	23,415
„ „ 20 „	6,252	7,148	6,774	420	454	26,910	190	499	29,840
„ „ 50 „	10,431	11,644	10,873	404	260	28,471	254	190	32,220

[1] Still slightly turbid after standing 20 hours.
[2] Ditto after standing 30 hours.

Finely Sifted and Sterilised Aluminium Oxide

Temperature at which vessels were kept = 9° C

—	Control Vessel			Experimental Vessel No 1 [1]			Experimental Vessel No. 2 [2]		
Before treatment . .	5,179 Bact. in 1 c.c.			4,598 Bact. in 1 c.c.			5,234 Bact. in 1 c.c.		
Aluminium oxide added	0			0·5 grm. per litre			1·5 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	5,729	5,116	5,356	970	820	35,622	488	640	32,689
„ „ 20 „	5,981	6,123	5,849	860	720	37,125	313	282	44,720
„ „ 50 „	6,014	7,433	6,630	670	1,440	33,057	248	260	47,010

[1] Still slightly turbid after 20 hours. [2] Still slightly turbid after 30 hours.

Pulverised and Sterilised Brick

Temperature at which vessels were kept = 7° C.

—	Control Vessel			Experimental Vessel No. 1 [1]			Experimental Vessel No. 2 [2]		
Before treatment . .	5,000 bact. in 1 c c.			4,740 Bact. in 1 c.c.			4,460 Bact. in 1 c.c.		
Pulverised brick added	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of Water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	5,610	4,823	4,764	475	575	21,080	240	375	36,070
„ „ 20 „	6,743	5,210	5,347	372	353	128,280	72	43	198,360
„ „ 50 „	5,984	6,991	7,200	2,670	2,860	242,680	1,673	1,945	236,420

[1] Liquid was clear after 2½ hours. [2] Liquid was almost clear after 7½ hours.

Pulverised and Finely Sifted Sterilised Vegetable Charcoal

Temperature at which vessels were kept = 7° C.

—	Control Vessel			Experimental Vessel No. 1 [1]			Experimental Vessel No. 2 [2]		
Before treatment . .	5,720 Bact. in 1 c.c.			4,892 Bact. in 1 c.c.			6,023 Bact. in 1 c.c.		
Charcoal added . .	0			0·5 grm. per litre			2 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	5,134	4,919	5,064	890	1,002	38,720	719	934	50,940
„ „ 20 „	7,640	6,134	5,030	320	230	105,630	180	190	235,610
„ „ 50 „	7,851	8,915	7,434	538	670	107,840	481	530	240,400

[1] Slightly turbid after 20 hours. [2] Slightly turbid after 30 hours.

Pulverised and Finely Sifted Sterilised Coke

Temperature at which vessels were kept = 7° C.

—	Control Vessel			Experimental Vessel No. 1[1]			Experimental Vessel No. 2[1]		
Before treatment .	7,119 Bact. in 1 c.c.			6,548 Bact. in 1 c.c.			7,069 Bact. in 1 c.c		
Coke added . . .	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 1 hour	7,184	6,870	6,934	5,374	4,835	6,719	6,274	6,375	7,140
„ „ 6 hours	6,984	7,230	6,952	3,788	3,675	8,760	3,133	3,660	14,907

[1] In both cases after standing half an hour the water was clear.

Dry, White, Finely Sifted, and Sterilised Sand

Temperature at which vessels were kept = 7° C.

—	Control Vessel			Experimental Vessel No. 1[1]			Experimental Vessel No. 2[1]		
Before treatment . .	5,180 Bact. in 1 c.c.			5,616 Bact. in 1 c.c.			5,279 Bact. in 1 c.c.		
Sand added . . .	0			0·5 grm. per litre			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 1 hour	5,276	4,993	6,040	4,848	4,684	5,066	3,234	4,998	6,118
„ „ 6 hours	5,047	5,150	6,101	3,784	4,315	6,988	2,122	2,045	9,336

[1] After from three to seven minutes the sand had in both cases fallen to the bottom.

In the above experiments insoluble substances were used exercising no chemical action either on the water or on the bacteria; in order to ascertain whether the results of sedimentation would be influenced by more or less soluble substances acting chemically on the water, and doubtless also on the bacteria, Krüger made the following further investigations:—

Finely Sifted and Sterile Magnesium Oxide

Temperature at which vessels were kept = 7° C.

—	Control Vessel			Experimental Vessel No. 1[1]			Experimental Vessel No. 2[2]		
Before treatment . .	4,140 Bact. in 1 c.c.			4,676 Bact. in 1 c.c.			4,120 Bact. in 1 c.c.		
Magnesium oxide added	0			0·5 grm. per litre			1·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2 hours	4,260	4,014	4,316	1,414	1,410	16,436	970	1,000	20,541
„ „ 20 „	5,013	4,670	4,814	694	695	11,065	680	432	7,772
„ „ 50 „	5,139	4,067	5,432	846	900	5,040	860	1,950	2,781

[1] The water became clear after standing between 20 and 30 hours.
[2] The water was rendered alkaline by the addition of the magnesium oxide.

Finely Sifted and Sterilised Hard-wood Ash

The temperature in both cases was 11° C.

—	Control Vessel			Experimental Vessel		
Before treatment .	18,368 Bact. in 1 c.c.			18,629 Bact. in 1 c.c.		
Wood-ash added .	0			2·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom
After standing 15 hrs. .	42,737	45,388	54,268	30	24	4,600
	Again					
Before treatment .	3,875 Bact. in 1 c.c.			2,178 Bact. in 1 c.c.		
Wood-ash added . .	0			1·0 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom
After standing 22 hrs. .	39,530	35,547	31,221	833	4,330	73,961

NOTE.—The water, after agitation with the wood-ash, acquired of course an alkaline reaction. It would appear that this alkalinity, which was more especially marked in the lowest layers, acted prejudicially upon the bacteria, inasmuch as in the first experiment there were very many fewer organisms found at the bottom of the flask containing the treated than the untreated water, whilst in the second experiment, when less wood-ash was added and the alkalinity was therefore much less marked, the numbers at the bottom were not diminished, but greatly increased. The results are obviously in neither experiment solely attributable to the mechanical effect produced by sedimentation, but are complicated by the chemical effect of the soluble ingredients of the wood-ash.

Lime

Temperature at which vessels were kept = 8° C.

—	Control Vessel			Experimental Vessel		
Before treatment .	4,978 Bact. in 1 c.c.			59,576 Bact. in 1 c.c.		
Lime added . .	0			0·2 grm. per litre		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom
At once . . .	5,128	4,984	5,315	878	763	346
After standing 2 days and 19 hrs. . .	20,521	19,577	23,603	634	1,684	153
After standing 22 days and 18 hrs. . .	11,002	12,320	23,820	2,729	836	1,176

After 42 hours the water was perfectly clear.

The lime was not sterilised, but was slaked and mixed with water, the resulting milk of lime being then added to the experimental vessel. The water became alkaline after the addition of the lime, and whilst this alkalinity at the bottom of the vessel was very strong after a time, in the middle and upper layers it decreased the longer the water remained standing.

It is unfortunate that the author does not give any particulars concerning the chemical composition of the water operated upon. It is, however, pretty obvious, from the strongly alkaline reaction of the water after

treatment, that the quantity of lime added was altogether in excess of that required for the removal of the 'temporary hardness' by Clark's Process, and the results are, therefore, in no way indicative of what takes place in that classical method of water purification; it is in fact rather the proportion in which lime is added in the chemical treatment of sewage, and it is obvious that in the experiment the lime has acted as a bactericide, and not as a simple precipitant, as there are fewer microbes at the bottom than at the top of the treated water.

Lime and Crude Sulphate of Alumina

Temperature at which vessels were kept = 12° C.

—	Control Vessel			Experimental Vessel [1]		
Before treatment .	2,265 Bact. in 1 c.c.			2,010 Bact. in 1 c.c.		
Lime and crude sulphate of alumina added .	0			0·2 grm. per litre [2]		
Layer of water examined	Top	Middle	Bottom	Top	Middle	Bottom
After standing 2½ hrs.	2,347	2,100	2,490	220	254	171
,, ,, 26 ,,	15,872	18,460	23,760	448	316	6,462
,, ,, 48 ,,	68,865	44,691	78,813	950	1,306	791

[1] The water was clear after standing 26 hours, and increased in alkalinity during standing. The amount of material used in the above experiments was less than in the other cases, in order to represent as nearly as possible the quantity which is used on a large scale for the purification of sewage. Thus in Halle to every 1,000 cb.m. of water there are added 40 kilos. of crude sulphate of alumina, and 150 kilos. of slaked lime, a proportion closely resembling that used above.

[2] ·15 grm. lime, ·05 crude sulphate of alumina.

In comparing Krüger's results with those previously obtained by one of us, it should be noted that in our experiments with insoluble solid particles (coke, vegetable and animal charcoal, chalk, &c.) we purposely employed large proportions of these materials (20 grms. per litre of water), in order that any power they might possess of attracting bacteria during their subsidence should be fully manifested, whilst in Krüger's experiments the proportions of these materials used are very much smaller (·5–2·0 grms. per litre), and in consequence the reductions in the number of micro-organisms observed

are generally much less striking. On the other hand, in experimenting with the soluble chemical precipitant lime, we carefully confined our attention to its use in such proportions as are employed in the actual treatment of water by Clark's Process, whilst Krüger has employed larger doses, in which bactericidal effect is superimposed on mere precipitation, with the result that, of course, much greater reductions in the number of micro-organisms were obtained.

Some further experiments on the subject of sedimentation have more recently been made by Messrs. V. and A. Babes;[1] but as their investigations are for the most part a repetition of those already made by other authors, it will not be necessary to deal with them in detail. It has long been known that alum has a remarkable purifying action upon water, and it is employed in various systems for water purification, such as the Hyatt, the American, &c.; it has also been shown by Leeds[2] to be very efficacious in the removal of bacteria. This investigator found that by the addition of one half-grain of alum to a gallon (·007 grm. per litre) of water the number of micro-organisms was reduced from 8,100 in 1 c.c. of untreated water to 80.

In the experiments by V. and A. Babes much larger quantities of material were used, as will be seen by reference to the following table :—

Bacterial Purification of Water by means of Alum
(V. and A. Babes)

	Powdered alum			Microbes in 1 c.c.
Untreated water contained				1,200
To 1 litre was added	0·25 g.	contained after standing	12 hours	0
,, ,,	0·2	,, ,,	,,	0
,, ,,	0·15	,, ,,	,,	0
,, ,,	0·1	,, ,,	,,	a few micro-organisms.

[1] 'Ueber ein Verfahren, keimfreies Wasser zu gewinnen,' by V. and A. Babes, *Centralblatt f. Bakteriologie*, vol. xii., p. 132, 1892.

[2] *Potable Water*, Floyd Davis, Boston, 1891, p. 86.

The treated water was examined again after 18, 24, 48, and 72 hours, and again after 4 days; and bacteria were only found (3–15 in a c.c.) in the water treated with 0·15 g. alum. The water was quite clear after 12 hours. The temperature of the room in which the vessels were kept varied from 8–15°C.

In order to ascertain in what manner the microbes might be distributed throughout the layers of water thus treated, a high cylindrical vessel (60 cm.) was filled with water, and 0·4 g. alum added to every litre.

Water taken from the surface after standing 24 hours contained about 20 microbes in 1 c.c., whilst in other similar investigations none were found. The water was also found to be quite sterile at a depth of 10, 20, 30 and 40 cm., and even after standing 4 days no organisms were discoverable.

It would appear that alum acts very deleteriously on water-bacteria, for whilst the untreated water contained, after standing for four days, 1,500 microbes in 1 c.c., and 6,000 microbes in 1 c.c. of the sediment, the sediment of the alum-treated water contained only 20–100 bacteria in a c.c. This would account for no subsequent redistribution of the microbes taking place in the upper layers. In consequence of the good results obtained by the use of alum, these authors have devised a piece of apparatus in which precipitation by means of alum, or other substances, is made to take place, and the treated water subsequently drawn off.

Lankester[1] has also experimented on this method of water purification, using ·25 grm. of alum to 1 litre of water; the untreated water gave, after standing 24

[1] Evidence given before the Royal Commission on the Metropolitan Water Supply, November 1892.

hours, 15,130 microbes in a c.c., whilst the treated water was found after a similar period of rest to be sterile. In another similar experiment the untreated and treated waters gave respectively 2,380 and 8 microbes in a c.c.

Effect of chemical precipitation on the bacterial contents of sewage.—For the most complete account of the removal of micro-organisms from sewage by the use of various precipitants, we must again turn to the masterly work emanating from the State Board of Health of Massachusetts.[1] In these investigations the sewage was allowed to run into a tank, where it was thoroughly stirred up, and from it a series of barrels were filled, to each of which the particular chemicals under examination were then added as desired. One barrel was always left to settle without the addition of any chemicals, to serve for comparison. The barrels were 30 inches high and held about 50 gallons each. After the sewage in each barrel had been thoroughly mixed with the chemicals added, it was allowed to settle, and a sample of the effluent above the sludge was then drawn from a tap situated about 10 inches from the bottom. The time of settling allowed in the following experiments was one hour.

Bacterial purification of Sewage with different amounts of Lime

	Number of bacteria in 1 c.c.
Original sewage	196,000
Ditto after settling 1 hour without additions . . .	123,200
Effluent with 600 lbs. of lime per 1 million gallons of sewage .	95,000
„ 800 „ „ „ „	65,000
„ 1,000 „ „ „ „	50,000
„ 1,500 „ „ „ „	12,180
„ 2,000 „ „ „ „	3,150

[1] *Experimental Investigations by the State Board of Health of Massachusetts*, Part II. p. 737, 1888–1890.

Lime continued

	Number of bacteria in 1 c.c
Original sewage	1,572,000
Ditto after settling 1 hour without additions	783,200
Effluent with 1,000 lbs. of lime per 1 million gallons of sewage	55,200
„ 1,200 „ „ „ „	49,500
„ 1,400 „ „ „ „	44,000
„ 1,600 „ „ „ „	11,448
„ 1,800 „ „ „ „	12,300
„ 2,000 „ „ „ „	5,920

Lime continued

	Number of bacteria in 1 c.c.
Original Sewage	1,364,000
Ditto after settling 1 hour without additions	1,440,000
Effluent with 1,000 lbs. of lime per 1 million gallons of sewage	71,000
„ 1,200 „ „ „ „	49,500
„ 1,400 „ „ „ „	24,208
„ 1,600 „ „ „ „	21,120
„ 1,800 „ „ „ „	7,384
„ 2,000 „ „ „ „	1,484

Copperas and lime.—In order to ascertain the value of copperas, or ferrous sulphate, the following experiments were conducted. As it had been found that the best *chemical* results were obtained when lime was used in conjunction with copperas, it was decided to try to establish the right proportions in which these materials should be added, and also to ascertain whether it is more advantageous that the sewage should be first mixed with the lime or the copperas respectively.

Bacterial purification of Sewage with different quantities of Lime and Copperas

Series A.—500 lbs. Copperas with different amounts of Lime

	Number of bacteria in 1 c.c.
Original sewage	176,000
Ditto after settling 1 hour without additions	126,500
lb. copperas Effluent with 500 per 1 million gallons of sewage	24,500
lb. lime „ „ and 200 per 1 million gallons of sewage	56,000
„ „ 400 „ „ „	72,000
„ „ 800 „ „ „	56,000
„ „ 1,200 „ „ „	16,128
„ „ 2,000 „ „ „	10,176

Series B.—Different proportions of Copperas with the corresponding proportions of Lime

	lb. copperas	lb. lime				Number of bacteria in 1 c.c.
Original sewage						322,400
Ditto after settling 1 hour without additions						271,400
Effluent with	100	530 [1]	per	1 million	gallons of sewage	20,620
„	200	580	„	„	„	16,640
„	400	630	„	„	„	7,370
„	700	720	„	„	„	9,204
„	1,000	800	„	„	„	6,972
„	2,000	1,100	„	„	„	1,269

In both the above series of experiments the lime was added to the sewage first, and thoroughly incorporated with it before the addition of the copperas.

In order to see if a better result could be obtained by mixing the sewage with the copperas first, and then adding the lime, or if as good a result could be obtained by precipitating the copperas with lime, and adding the precipitated ferrous hydrate to the sewage, the following experiments were made :—

Bacterial purification of Sewage with the same amounts of Copperas and Lime added in different ways

	Number of bacteria in 1 c.c.
Original sewage	507,800
Ditto after settling 1 hour without additions	422,400
Effluent with 500 lbs. of copperas and 650 lbs. of lime per million gallons of sewage; copperas added first	15,950
Ditto, lime added first	15,800
Effluent with 1,000 lbs. of copperas and 300 lbs. of lime per million gallons of sewage; lime and copperas added together	9,476
Effluent with 1,000 lbs. of copperas and 800 lbs. of lime per million gallons of sewage; lime and copperas added together	12,576
Ditto, copperas added first	9,944
Ditto, lime „ „	10,406

[1] It should be noted that in this sewage 500 lbs. of lime were required to combine with the free carbonic acid, whilst the additional quantity of lime employed corresponds to the sulphuric acid in the copperas added, and thus serves to decompose the latter.

Ferric salts.—Ferric salts were next investigated, both alone and when used in conjunction with lime. The following tables show the results obtained :—

Bacterial purification of Sewage with different amounts of Ferric Sulphate with and without Lime

Series A

	Ferric oxide			Number of bacteria in 1 c.c.
Original sewage				218,960
Ditto after settling 1 hour without additions				132,480
Effluent with	200 lbs.	per million gallons of sewage		30,442
,,	,,	and 400 lbs. of lime per million gallons of sewage		49,025
,,	,,	800	,, ,, ,, ,,	22,000
,,	400	0	,, ,, ,, ,,	6,080
,,	,,	500	,, ,, ,, ,,	9,800
,,	,,	1,000	,, ,, ,, ,,	8,940

Series B

				Number of bacteria in 1 c.c.
Original sewage				218,960
Ditto, after settling 1 hour without additions				132,480
Effluent with	400 lbs.	of ferric oxide per million gallons of sewage		6,080
,,	,,	,, and 500 lbs. of lime per million gallons of sewage		9,800
,,	,,	,, 1,000	,, ,,	8,940

Series C

				Number of bacteria in 1 c.c.
Original sewage				1,398,600
Ditto after settling for 1 hour without additions				970,000
Effluent with	100 lbs.	of ferric oxide per million gallons of sewage		223,432
,,	200	,, ,, ,, ,, ,,		204,508
,,	300	,, ,, ,, ,, ,,		130,118
,,	400	,, ,, ,, ,, ,,		66,528
,,	300	,, and 1,000 lbs. of lime per million gallons of sewage; lime mixed with sewage first		176,904
,,	300 lbs.	of ferric oxide and 1,000 lbs. of lime per million gallons of sewage; ferric oxide mixed with sewage first.		46,445

Alum.—The action of aluminium sulphate, or crude alum, was next tried alone and in conjunction with lime, with the following results :—

Bacterial purification of Sewage with Alum and Lime

Series A.—1,000 *lbs. of Alum and different amounts of Lime*

	Number of bacteria in 1 c.c.
Original sewage	835,200
Ditto after settling 1 hour without additions	994,500
Effluent with 1,000 lbs. of alum per million gallons of sewage	7,200
„ „ „ „ „ „ „ and 400 lbs. of lime	54,280
Effluent with 1,000 lbs. of alum per million gallons of sewage and 600 lbs. of lime	42,090
Effluent with 1,000 lbs. of alum per million gallons of sewage and 800 lbs. of lime	25,872
Effluent with 1,000 lbs. of alum per million gallons of sewage and 1,000 lbs. of lime	19,236
Effluent with 1,000 lbs. of alum per million gallons of sewage and 2,000 lbs. of lime	520

Series B.—Using different amounts of Alum and Lime

	Number of bacteria in 1 c.c.
Original sewage	361,328
Ditto after settling 1 hour without additions	347,600
Effluent with 500 lbs. of alum per million gallons of sewage	11,160
„ „ „ and 400 lbs. of lime per million gallons of sewage	92,250
Effluent with 500 lbs. of alum and 800 lbs. of lime per million gallons of sewage	31,375
Effluent with 1,000 lbs. of alum alone per million gallons of sewage	63,750
Effluent with 1,000 lbs. of alum and 500 lbs. of lime per million gallons of sewage	7,900
Effluent with 1,000 lbs. of alum and 1,000 lbs. of lime per million gallons of sewage	4,760

The above tables show that a particular proportion of lime, depending of course on the nature of the sewage, gives as good or a better result than either more or less; and again that, speaking generally, the more copperas, the more ferric sulphate, and the more alum employed, the better is the result achieved. Moreover, whilst ferric sulphate and alum usually require no lime for complete precipitation, the latter is only

secured in the case of copperas when lime is also added.

In the following table are finally recorded the results of experiments made to ascertain the relative efficiency of the several chemicals in purifying one and the same sample of sewage. In this series the most advantageous proportion of lime was employed, and the other chemicals were applied under the conditions found in previous experiments to be the most favourable, whilst the actual amounts used were so selected as to be of the same pecuniary value as that of the lime.

Results of treatment of Sewage with equal money-values of different Chemicals

—	Cost of chemicals in cents.[1]	Number of bacteria in 1 c.c.	Reduction per cent.
Original sewage	—	25,840	—
Ditto after settling 1 hour without additions .	—	10,920	—
Effluent with 1,800 lbs. of lime per million gallons of sewage	30	1,911	93
Effluent with 1,000 lbs. of copperas and 700 lbs. of lime per million gallons of sewage .	30	16,044	38
Effluent with 270 lbs. of ferric oxide per million gallons of sewage	30	2,047	92
Effluent with 650 lbs. of alum per million gallons of sewage	30	2,475	91
Effluent with 360 lbs. of ferric oxide per million gallons of sewage . . .	40	1,980	93
Effluent with 870 lbs. of alum per million gallons of sewage	40	1,800	93

[1] Per inhabitant annually, calculated on the assumption that 100 gallons of sewage daily are produced by each individual. It should be noted that American sewage is generally very much more dilute than that to which we are accustomed in British towns.

In connection with these interesting results obtained by the Massachusetts Board, we may appropriately append the following general conclusions from their Report of 1890, p. 788 :—'When a nuisance is produced by sewage in any way, the direct cause is usually the development of organisms fed by the organic matter and nitrogen compounds of the sewage. To secure the

absence of organisms in any pond or stream where food is present is a hopeless task. It thus happens that, while the organisms are the real cause of the trouble, their removal from sewage is often of less importance than the removal of the matter in the sewage on which they feed. The proportion of organic matter removed does not necessarily represent the proportion of food for organisms removed, for some kinds of organic matter are no more suitable food for bacteria than is saw-dust for horses. An effluent from a sewage filter, where nitrification is complete, containing 2 per cent. of the total organic matter of the sewage, will not serve as food for bacteria, because it has been worked over already by bacteria in the filter, and nearly everything available has been removed. If, on the other hand, sewage is mixed with fifty times its volume of pure water, so that it contains the same amount of organic matter as the effluent, the bacteria will increase enormously for a few days. From this point of view, the effluent is many times purer than is indicated by the ratio of its organic matter to that of the sewage.

'With sewage precipitation the case is entirely different, for here there is no bacterial action. There is, however, some reason to think that the organic matter left is not so good a food, and therefore not so dangerous as that removed. Sewage settled alone will keep turbid with organisms, and in a day or two masses of zoogloea (dead or resting bacteria) separate from it. Sewage precipitated by either copperas, ferric sulphate, or alum in suitable quantities, has repeatedly remained so clear that the bottom of the barrels could be distinctly seen through more than two feet of liquid for one or two weeks. In these cases no flakes of zoogloea so characteristic of untreated sewage have been seen, and the odour is much less than that of sewage alone.

'This question of the quality of the organic matter left by precipitation has not been sufficiently investigated, but the indications are, that it is more objectionable than the same amount in the effluents from sewage filtration through sand, but less objectionable than that in sewage.

'When untreated sewage is put into a small stream or pond, it often happens that the suspended matters settle out, forming considerable deposits, which, putrefying out of contact of the air, give rise to very offensive gases. It is hardly probable that well-precipitated sewage would do this, for almost no suspended organic matter is present when it leaves the settling tank, and very little soluble matter is precipitated on exposure to the air.

'Another nuisance which might be caused by putting precipitated sewage into a stream or pond is the growth of algae—green plants fed by the ammonia of the sewage. It may be said, however, that this growth would be no greater than that caused by the crude sewage, and probably not much greater than that caused by filtered sewage; for, in the latter case, while the ammonia is removed, nearly an equivalent of nitrate is formed, and this serves as food for algae almost as readily as ammonia. A number of fishes were put into precipitated sewage. In each case the fish died within five minutes. This sudden death cannot be due to the chemicals used, for it was found that the fishes lived for a considerable time in solutions of the chemicals much stronger than those present in the sewage. The fishes died for want of air. Sewage contains no dissolved oxygen, and, if any is absorbed from the air, it is quickly taken up by the organic matter. The precipitated sewage also contains no oxygen.

'Using lime as a precipitant, we have found that

there is a certain definite amount of lime, depending upon the composition of the sewage, which gives a better result than less, and as good or a better result than more. This amount of lime is that which exactly suffices to form normal carbonate with all the carbonic acid of the sewage. It is possible in a few minutes, by simple titration, to determine approximately the amount of uncombined carbonic acid present in sewage, and how much lime will be required to combine with it. It is also possible to determine in a similar way, after mixing, whether enough or too much lime has been added. The amount of lime required by Lawrence sewage averages about 1,600 pounds per million gallons.

'Ordinary house-sewage is not sufficiently alkaline to precipitate copperas, and a small amount of lime must be added to obtain good results. The quantity of lime required depends both upon the composition of the sewage and the amount of copperas used, and can be calculated from titration of the sewage. Very imperfect results are obtained with too little lime, and when too much is used the excess is wasted, the result being the same as with a smaller quantity.

'After mixing the sewage with both copperas and lime, if enough or too much lime has been used the mixture will colour phenolphthaleïn red, while, if too little has been used, no colour will be produced. This test can conveniently be used by people having no knowledge of chemistry, and affords an easy and very accurate method of applying enough lime, and of avoiding a useless excess.

'Using in each case a suitable amount of lime, the more copperas used the better the result; but, with more than one-half a ton per million gallons, the improvement does not compare with the increased cost.

'Some acid sewages contain a considerable amount of iron in solution, and in these cases precipitation by lime is really the rendering available of the copperas already in the sewage, and so is properly classed as an iron treatment rather than a lime treatment. In this case the reaction with phenolphthaleïn shows the presence of enough lime.

'In precipitation by ferric sulphate and crude alum the addition of lime was found unnecessary, as ordinary sewage contains enough alkali to decompose these salts. Within reasonable limits the more of these precipitants used the better is the result, but with very large quantities the improvement does not compare with the increased cost.

'Using equal values of the different precipitants, applied under the most favourable conditions for each, upon the same sewage, the best results were obtained with ferric sulphate. Nearly as good results were obtained with copperas and lime used together, while lime and alum each gave somewhat inferior effluents.[1] The range of these results was, however, comparatively narrow; and it may be that, with sewage of a different character, or with variations in the prices of the chemicals, it would be advantageous to use copperas with lime, or even alum. When lime is used there is always so much lime left in solution that it is doubtful if its use would ever be found satisfactory except in case of acid sewage.

'It is quite impossible to obtain effluents by chemical precipitation which will compare in organic purity with those obtained by intermittent filtration through sand.

'It is possible to remove from one-half to two-thirds

[1] These remarks refer to the soluble organic matter removed by the several chemicals, and it will be seen from the table on p. 210 that these precipitants do not by any means stand in the same order as regards their relative power of removing bacteria.

of the soluble organic matter of sewage by precipitation with a proper amount of an iron or aluminium salt, and it seems probable that, in some cases at least, if the process is carried out with the same care as is required in the purification of sewage by intermittent filtration, a result may be obtained which will effectually prevent a public nuisance.'

The experiments of all the above investigators, therefore, clearly prove that the most remarkable removal of micro-organisms from water may take place by means of precipitation alone. This precipitation may be of three different kinds :—

1. In which the precipitant is added in a solid and insoluble form, and must therefore be practically inert from a chemical point of view. To this class belong sand, china-clay, infusorial earth, chalk, vegetable and animal charcoal, coke, &c. In the case of the various forms of carbon, more especially in that of animal charcoal, a small chemical effect is doubtless exerted on some of the constituents of the water ; there is, however, no evidence that they have any bactericidal power, but rather the reverse. It will be noticed that there is the greatest diversity in the bacterial purification effected by the substances belonging to this class, the more porous and slowly subsiding substances carrying with them a much larger proportion of the suspended bacteria than those which are smooth, impervious, compact, and therefore rapidly subsiding.

This class of precipitation is particularly interesting, inasmuch as it is of the kind which takes place during the storage of turbid water in large reservoirs or lakes. As would be expected, of course, the bacterial improvement is the more striking the larger the quantity of material added, as in this way the points of attraction for the bacteria are correspondingly multiplied.

2. In which the precipitant is soluble and more or less chemically active, but in which, through entering into reaction with constituents of the water, it is so rapidly converted into an insoluble form that little or no directly bactericidal effect can be exerted.

To this class of precipitants belongs lime when used in suitable proportions for the softening of water containing 'temporary hardness' in Clark's Process, but not when employed in excess, as in the latter case, of course, a directly bactericidal effect will be produced by the caustic lime remaining in solution.

Alum again, when used in excessively minute quantities, may be regarded as belonging to this class of precipitants.

The precipitates in these cases, being actually generated within the water containing bacteria, are extremely successful in entangling the latter and dragging them to the bottom. Moreover, in this respect the bulky gelatinous precipitate of alumina is naturally more effective than the pulverulent or even crystalline precipitate of carbonate of lime.

3. In which the precipitant is soluble, chemically active, and added in such quantity that a direct bactericidal effect is produced in addition to the mechanical one of precipitation. This kind of treatment cannot as a rule be employed for the purification of drinking water, but is abundantly made use of in the chemical treatment of sewage.

CHAPTER VI

ON THE MULTIPLICATION OF MICRO-ORGANISMS

One of the first difficulties which the water-bacteriologist had to encounter was the discovery that if any considerable interval is permitted to elapse between the collection of a sample of water and its subsequent examination, the number of bacteria present is generally found to have undergone extensive multiplication. This disturbing factor in the accurate appreciation of the normal bacterial contents of a given water was not discovered until some time after the introduction and application of Koch's gelatine-plate process to the investigation of water; indeed, it was not unnaturally a matter of great surprise to find that some micro-organisms are capable of multiplying largely in waters of great organic purity, and even in ordinary distilled water itself.

One of the first recorded observations on this subject was made by one of us[1] in 1885. Three sterilised Winchester bottles were filled with ordinary distilled water, and a few drops of diluted urine water added; the bottles were then plugged with sterilised cotton-wool, placed in a room (temperature about 10° C.) and left at perfect rest. The following were the results obtained:—

[1] 'Removal of Micro-organisms from Water,' Percy Frankland. *Proc. Roy. Soc.* 1885.

Hours	Number of Micro-organisms in 1 c.c. of water
0	1,073
6	6,028
24	7,262
48	48,100

Thus an enormous multiplication of the bacteria introduced was found to take place in distilled water in which the amount of organic matter present must have been quite a vanishing quantity.

Leone[1] published in the following year some observations on the effect of preserving the Mangfall water supplied to Munich in sterilised flasks and examining the sample on successive days. The results obtained were :—

	Number of Micro-organisms in 1 c.c. of water
Water at time of collection contained	5
Ditto after standing 24 hours in sterilised flask	100
„ „ 2 days „ „	10,500
„ „ 3 „ „	67,000
„ „ 4 „ „	315,000
„ „ 5 „ „	more than ½ million

In these and in similar experiments conducted by Meade Bolton and Heraeus[2] an enormous increase in the number of micro-organisms is seen to take place during the period over which the observations extended. Cramer[3] examined, however, some samples of water from the Lake of Zürich after they had been standing for different periods of time up to 70 days, and made the important discovery that, although at first the bacteria multiply extensively, a point is reached when the numbers begin to decline, thus :—

[1] Leone, 'Sui microorganismi delle acque potabili; loro vita nelle acque carboniche.' *Archiv für Hygiene*, 4 Band, 2 Heft, 1886, p. 168.

[2] 'Ueber das Verhalten der Bacterien im Brunnenwasser,' *Zeitschrift für Hygiene*, vol. i. p. 208, 1886.

[3] *Die Wasserversorgung von Zürich und ihr Zusammenhang mit der Typhusepidemie des Jahres* 1884, Zürich, 1885, p. 91.

Hours and days during which the water was preserved	Number of Micro-organisms in 1 c.c. of water
0 hours	143
24 „	12,457
3 days	328,543
8 „	233,452
17 „	17,436
70 „	2,500

Miquel[1] has extended these observations in an interesting manner by keeping a bottle of river Seine water shut up for nine years, and whilst at the time of collection 4,800 bacteria per c.c. were found, at the end of the nine years there were only 220 discoverable. Again, a sample of Vanne water, containing at the time of collection 66 organisms per c.c., at the end of 10 years was found to be absolutely sterile.

In 1886 a systematic series of experiments on the multiplication of micro-organisms was carried out by one of us[2] in this country, and by Meade Bolton[3] in Germany.

The waters experimented with in the first-mentioned series of investigations were the raw river-waters of the Thames collected at Hampton, and of the Lea at Chingford, as well as the same waters after sand-filtration, and as distributed by the water companies to London, besides the deep-well water derived from the chalk.

As we have already seen, the bacterial composition of these various waters is very different, and hence it was expected that the phenomenon of multiplication would likewise show marked deviations in the case of each class of water.

[1] *Revue d'Hygiène*, tom. ix. p. 737.

[2] 'On the Multiplication of Micro-organisms,' Percy Frankland. *Proc. Roy. Soc.* 1886.

[3] 'Ueber das Verhalten verschiedener Bacterienarten im Trinkwasser,' *Zeitschrift für Hygiene*, 1886, vol. i.

That this was the case the following experiments illustrate very clearly :—

Description	Number of bacteria in 1 c.c.		
	On day of collection, temp. of water 8° C.	After standing in dark at 20° C. for 2 days	Ditto after 4 days
River Thames at Hampton . . .	12,250	4,386	2,018
River Lea at Chingford . . .	7,300	2,148	1,286

The organisms in the raw river-water thus underwent a marked reduction after storing for two and four days respectively in stoppered bottles.[1] But if these waters are kept in the incubator at a high temperature (35° C.), the reverse takes place in the first instance, thus :—

Description	Day of collection	Standing 1 night in incubator at 35° C.	Standing for 8 days in incubator at 35° C.	Standing for 16 days in incubator at 35° C.
River Thames at Hampton	15,800	665,280	3,616	15,460

Thus a very rapid increase in the numbers present took place at first, but an exposure of eight days to

[1] It should be pointed out that in these experiments with unfiltered river-water the initial number of bacteria present was very large. We have subsequently found that when the initial numbers are smaller and the water is preserved in flasks to which there is free access of air through cotton-wool stoppers, the multiplication may be very extensive. This result is, moreover, in precise accordance with the experiences of Miquel on the water of the Seine, in which connection he remarks :—' De l'eau de la Seine, puisée à Ivry pendant l'été, d'une richesse inférieure à la moyenne annuelle, peut présenter des recrudescences de germes assez élevées ; elle montre à cet égard une tendance manifeste à se comporter comme les eaux de source ; mais que surviennent les crues, que sa teneur en microbes atteigne à l'analyse immédiate 20,000 et 30,000 bactéries par centimètre cube, cette faculté de s'infester spontanément disparaît, et cette eau impure ne peut devenir le siège de recrudescences microbiennes rapides et soudaines.'

this temperature brought about a marked decline, whilst subsequently again a slight increase was observed.

These figures are intelligible if we remember, in the first place, the large quantity of bacteria present in this raw river-water; secondly, the numerous different *varieties* which invariably are found in such descriptions of water. These different varieties have each an individuality of their own, and whilst some flourish luxuriantly at a high temperature, others are destroyed by it. Thus we find in the first twenty-four hours an enormous increase in those forms which the heat of the incubator has placed at the greatest advantage. In the course of this multiplication they may have, and doubtless have, elaborated products which act ultimately inimically upon themselves, so that the field is cleared for the subsequent development and multiplication of those forms which had previously remained dormant.

As regards the effect of a rise of temperature upon the bacteria in water, Krüger[1] has more recently (1889) found that in samples of water examined by him at Jena, and kept for twenty hours at 7° C., the numbers present increased 0·08 times, at 10° C. 4·8, and at 12° C. 5·3 times; it must not, however, be supposed that these factors are capable of any general application, but they serve to indicate in a concise form the multiplication phenomena which took place in a particular instance.

Experiments were then instituted to compare the powers of multiplication possessed by the bacteria in the raw river-water with those remaining in the same water after undergoing storage and filtration at the hands of the water companies.

For this purpose, samples were collected from the mains of the various companies and subsequently ex-

[1] *Zeitschrift für Hygiene*, vol. vii. p. 90.

amined. The following experiments are selected, and may be regarded as typical :—

Description	Number of colonies obtained from 1 c.c.		
	Day after collection	Standing 5 days in dark at 20° C.	Standing 27 days in dark at 20° C.
River Thames as supplied by the Grand Junction Co.	4,894	15,950	3,757
River Thames as supplied by the Lambeth Co.	2,587	6,980	2,749

The micro-organisms in these *filtered* waters multiply, therefore, at 20° C. with far greater rapidity than those in the unfiltered waters, which, as already pointed out, have rather a tendency to become diminished, unless raised to the temperature of the incubator. The following experiments again show the effect of placing these filtered waters in the incubator for one night :—

Description	Number of colonies obtained from 1 c.c.	
	Day of collection	Standing through night in incubator at 37° C.
River Thames as supplied by the Chelsea Co. .	305	62,483
Ditto Lambeth Co.	265	44,289
Ditto Grand Junction Co.	208	22,842

Thus, at the temperature of the incubator, the multiplication which takes place during a single night is enormous.

Experiments were also made to ascertain the effect of placing these filtered waters in a refrigerator for a week, and the following examples illustrate this point :—

Description	Number of colonies obtained from 1 c.c.	
	Day after collection	Standing 7 days in a refrigerator
River Thames as supplied by the Chelsea Co.	159	11,437
Ditto Grand Junction Co . . .	4,894	14,965

Thus the multiplication in these waters is very marked, even when preserved at the low temperature of a refrigerator, and clearly indicate the inadmissibility of delaying the bacteriological examination of a sample of water beyond a few hours, even if the sample be packed in ice.

An examination was then made of the deep-well water obtained from the chalk, which, as is well known, contains but the merest trace of organic matter, and presents, therefore, in this respect a marked contrast to the river-waters described above. The following results show that the power of multiplication possessed by the bacteria in this well-water is altogether enormous, thus :—

Description	Number of colonies obtained from 1 c.c.		
	Day after collection	Standing 3 days at 20° C. (dark)	Standing 16 days at 20° C. (dark)
Kent Well	96	178,379	51,843

Description	Number of colonies obtained from 1 c.c.		
	Day of collection	Standing 1 day at 20° C.	Standing 3 days at 20° C.
Kent Well	7	21	495,000

In considering these results, it must be remembered that at the outset this water is almost wholly free from

micro-organisms, and that it has never before been inhabited by such living matters ; it is only reasonable to infer, therefore, that those of its ingredients which are capable of nourishing the particular micro-organisms which flourish in it are wholly untouched, whilst in the case of the river-waters the most available food supply must have been largely explored by the numerous generations of micro-organisms which have inhabited them. Also far *fewer* varieties of micro-organisms are found in this deep-well water than in the case of the river-waters, hence those forms which are present will have a more undisputed field for multiplication in the absence of competing forms. This would also explain the greater capacity for multiplication which is exhibited by the filtered river-waters as compared with the water in its raw condition, a large number of varieties having been eliminated in the treatment which the water has undergone at the waterworks during storage and sand-filtration.

This remarkable phenomenon of bacterial multiplication, generally taking place more abundantly in pure or in waters containing a small number of microbes to start with than in impure or waters containing a large initial number, has been made the subject of some highly interesting and suggestive investigations by Miquel.[1]

Thus a sample of the Vanne spring-water, in which only 150 micro-organisms were present in 1 c.c. at the time of collection, on being kept for twenty-four hours at 20° C., contained as many as from 30,000 to 40,000, whilst a sample of the Ourcq canal-water, which is highly polluted and very rich in bacterial life to begin with, on standing for a similar length of time, exhibited no increase in the numbers present.

[1] *Manuel pratique d'Analyse bactériologique des Eaux*, Paris, 1891, p. 146.

The results obtained with different waters have been graphically brought together by Miquel in the following diagrams, in which the abscissae represent the duration of time, and the ordinates the number of

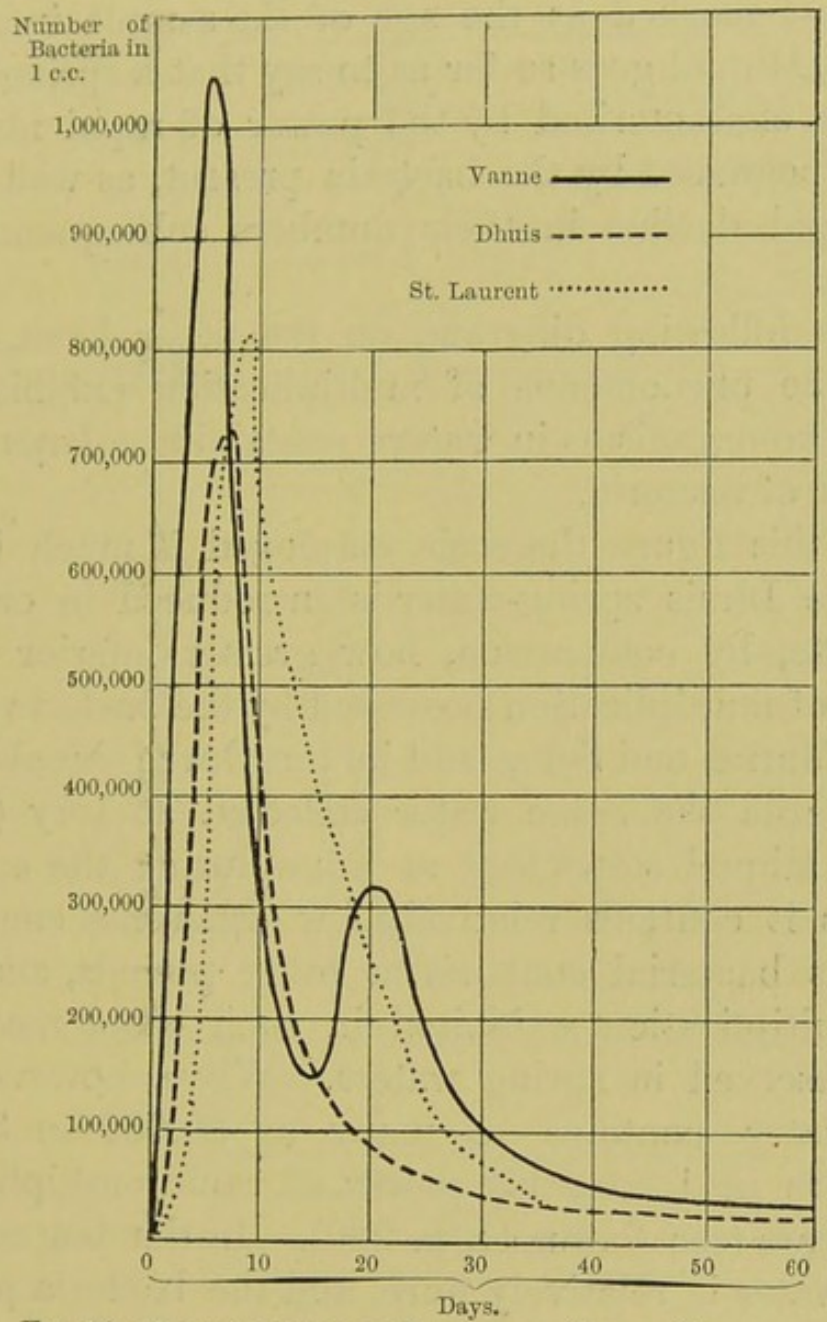


FIG. 16. MULTIPLICATION OF BACTERIA IN SPRING WATERS. (Miquel.)

bacteria present in 1 c.c. All the samples were maintained at from 29° to 30° C.

The above figure represents the behaviour of certain spring-waters containing initially only a *small* number

of microbes. Miquel points out how rapid and enormous the multiplication is which takes place in the first instance, and how, when once the maximum has been reached, the decline as rapidly follows, becoming, however, less marked as the age of the sample increases. In fact, Miquel goes so far as to say that a spring-water may be characterised by the power of rapid multiplication possessed by the bacteria present, as well as by the rapid decline in their numbers subsequently exhibited.

The following diagram, on the other hand, represents the phenomenon of multiplication exhibited by the micro-organisms in waters containing a *large* initial number of bacteria.

In this figure the scale employed is much larger, and the Dhuis spring-water is introduced in order to illustrate, by comparison, how greatly inferior is the power of multiplication possessed by the bacteria in the rivers Marne and Seine and in the Ourcq canal-water. As regards the Seine water collected at Ivry (above Paris), Miquel states that at times during the summer months it contains relatively few organisms compared with its bacterial contents at other periods, and that the multiplication exhibited in such cases resembles that observed in spring waters. When, however, the river-water contains as many as 20,000 to 30,000 microbes in 1 c.c., this power of rapid multiplication disappears (see foot-note, p. 220). In the diagram the Seine water is relatively pure, and the bacteria present behave, to a certain extent, like those in spring-water, only the multiplication is neither so rapid nor so extensive. The river Marne exhibits first a rise in the numbers present, then a decrease, followed by a subsequent increase, a phenomenon which is not unfrequently met with in the multiplication of bacteria in water. The

most interesting feature of all in this figure is the behaviour of the bacteria in Ourcq canal-water. This water, containing to start with about 8,000 organisms in 1 c.c., requires as long as twenty days before the numbers

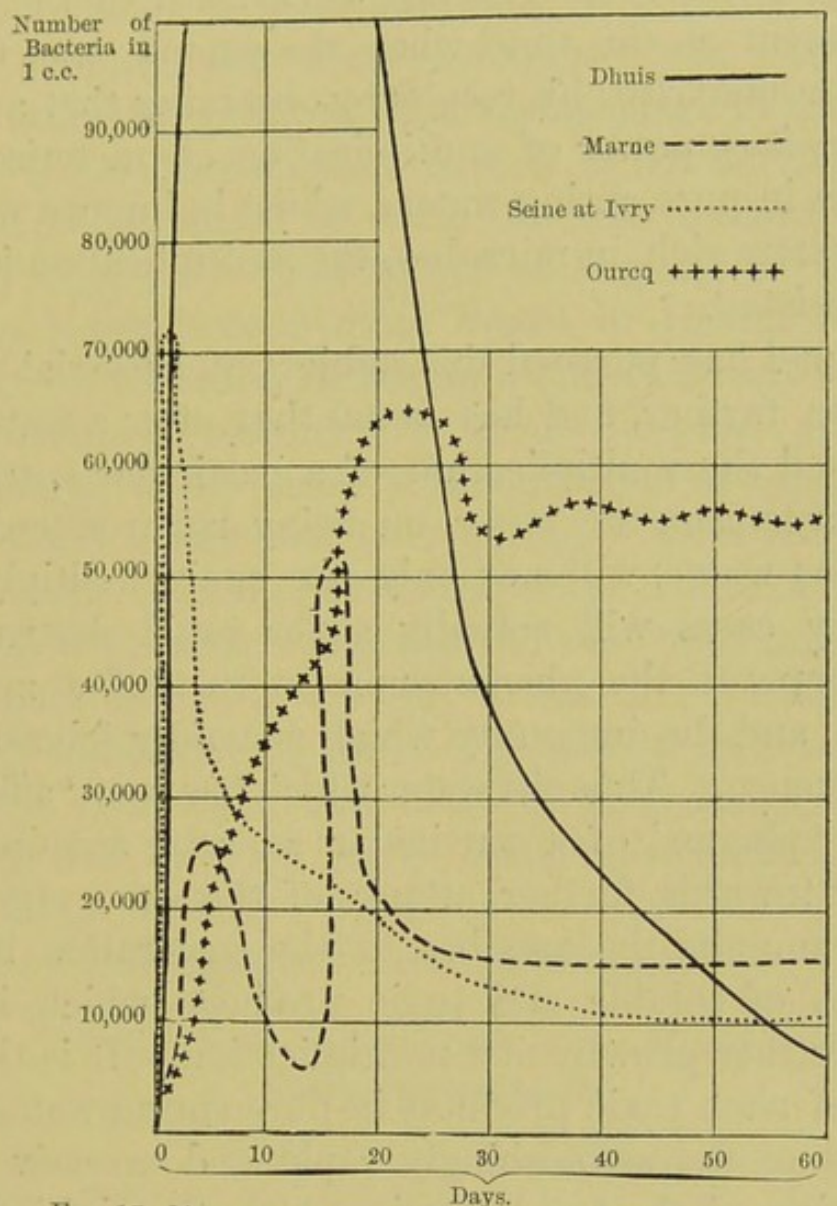


FIG. 17. MULTIPLICATION OF BACTERIA IN IMPURE WATERS. (Miquel.)

present reach 60,000, after which point a slight decrease is noticed; but the number of bacteria subsequently remains almost stationary at this high level, and examinations made of this sample at the end of six months, a year, and even after, revealed practically no alteration. Miquel goes on to say that the bacteria in the Seine

water exhibited at the end of a much shorter time a marked decline in their numbers, but after some months the decrease becomes more and more slow, until at the end of from ten to twelve years the number of bacteria is equal to, or is only a half or a third of the number present at the time when the sample was taken. Miquel summarises his results by observing that a *rapid but transitory* power of multiplication characterises the bacteria in pure spring-waters, whilst in impure waters, or in waters rich in microbes, the multiplication is *slow and persistent.*

Miquel has pursued this subject of bacterial multiplication further, and has found that after a water has supported the multiplication of a particular species of micro-organism, the latter, on being reintroduced into the same water, will not only not again multiply, but in many cases will actually suffer rapid destruction. He compares the phenomenon to that of a zymotic disease, and the immunity which generally follows as a consequence. Thus the water which has been 'afflicted' with a 'plague' of a particular microbe acquires immunity towards further attacks of the same organism. This immunity he ascribes to the generation by the bacteria of soluble and toxic products which inhibit their further growth and multiplication. It is the absence of such toxic products in pure spring-waters that permits of the astonishingly rapid and extensive multiplication of the few bacteria which they initially exhibit, and causes these waters to present such a marked contrast in this respect to more contaminated surface waters. These toxic products are destroyed on boiling, for a water which will not support any further bacterial multiplication acquires this property after boiling (for experimental confirmation of this statement, see p. 229). According to Miquel, however, these products, at any

rate those which are non-volatile, can be obtained in a concentrated form by evaporating a large volume (eight to ten litres) of the water containing them at a low temperature (30° to 35° C.); the concentrated solution which remains is then found to be actually toxic to some animals, and when introduced into waters prevents bacterial multiplication taking place in the latter.

It is very satisfactory to find independent experimental confirmation of some of these interesting and highly suggestive observations of Miquel's in the work of the Massachusetts State Board of Health. In the Report [1] of the latter, to which we have already referred, experiments are recorded in which the power of multiplication of water bacteria is contrasted in one and the same water before and after boiling. Thus a quart of water from the City Service pipe was boiled in a clean flask with a return condenser for one hour and so rendered sterile. After cooling, it was mixed with 10 per cent. of unboiled city water. A control flask was filled with the same water unboiled, and both flasks, covered merely with inverted sterile beakers, were kept at the same temperature.

The following are the results obtained :—

Boiled Water infected with Water Microbes
(Massachusetts Report)

Date	Number of bacteria in 1 c.c.	
	City water unboiled	City water boiled
May 14, 1890	196	3
„ 17 „	79	74,880
„ 21 „	31	162 864
„ 27 „	759	90,000
June 4 „	12	103,486
„ 7 „	—	63,140
„ 10 „	—	128,040
„ 13 „	—	530

[1] Report for 1890, p. 593.

Thus, whilst in the unboiled water the multiplication was quite insignificant, in the boiled water it was altogether enormous.

The above results, obtained with the unboiled city water, also illustrate the point, which we have before referred to more than once, viz. that bacterial multiplication in surface waters may, in some cases, either not take place at all, or only to a limited extent (see pp. 219–224).

Still more recently investigations have been made by Percy Frankland[1] on the power of multiplication possessed by the bacteria present in Loch Katrine water as supplied to Glasgow. This is, as is well known, a peaty, moorland water, the organic constituents of which are nearly all of vegetable origin, almost wholly destitute of mineral matters, and excessively soft, differing entirely, therefore, in character from the waters described above. Samples were placed in a refrigator, the temperature of which was 9° C., also in an incubator maintained at 19° C. The following table exhibits the rate of multiplication exhibited by these bacteria at the high and low temperature respectively:—

Loch Katrine Water at 19° *C.*

Date of examination	Number of micro-organisms per c.c.
On July 6, 1892, date of collection[1]	74
„ 8 „ „	785 (average of 4 examinations)
„ 12 „ „	42,537 „ „

Loch Katrine Water at 9° *C.*

Date of examination	Number of micro-organisms per c.c.
On July 6, 1892, date of collection[2]	74
„ 8 „ „	785 (average of 4 examinations)
„ 12 „ „	14,462 „ „ „

[1] From July 6 to July 8 the water remained in stoppered bottles at about 12° C.; on July 8 it was transferred to flasks plugged with cotton-wool, placed in the incubator, and kept at about 19° C. until July 12.

[2] In this series the water, instead of being placed in the incubator, was removed to a refrigerator, and kept at about 9° C. until July 12.

[1] 'Experiments on the Vitality and Virulence of Sporiferous Anthrax in Potable Waters,' *Proc. Roy. Soc.* 1893, p. 222.

Thus, again, is shown the immense power of multiplication possessed by the bacteria in this pure moorland water; the phenomenon being most marked in the case of those flasks kept in the incubator at 19° C.

The experiments conducted by Meade Bolton confirm also the rapid increase which takes place in water bacteria in the first instance, and the gradual decline which sets in afterwards. (See also Kraus's experiments with the Munich water, pp. 294 and 301.) It is difficult to ascertain what was the nature of the water experimented with, as beyond the words *Brunnen*, *Quell Wasser*, and *Wasserleitung* (*Quellwasser*) there is nothing whereby their character can be more accurately defined. Some exceedingly interesting experiments were, however, conducted by this author on the powers of multiplication possessed by particular water bacteria when introduced into sterilised potable water and sterilised distilled water respectively. For this purpose two organisms, the *Micrococcus aquatilis* (see p. 494) and the *Bacillus erythrosporus* (see p. 439), occurring very constantly in the water under examination, were isolated and introduced, both in large and small numbers, into sterilised samples of water, with the following results :—

Experiments with sterilised ordinary Water

—	Number of colonies obtained from 1 c.c.					
	At +1° C.			At +22° C.		
	Directly	After 48 hours	After 72 hours	Directly	After 48 hours	After 72 hours
Micrococcus aquatilis	800	3,420	820	—	Innumerable	Innumerable
	1,400	4,000	580			
Bac. erythrosporus, large quantity	Innumerable	60,000	—	Innumerable	,,	,,
		64,600				
Ditto, smaller quantity	4,020	5,000	2,700	3,400	,,	,,
	3,500	4,000	2,700	3,800		

Whether the number introduced was large or small the phenomenon of multiplication was observed, and in the case of those samples kept at +1° C. a subsequent decline took place, whilst the higher temperature appeared to favour very markedly the growth of these particular water bacteria, and during the time over which the observations extended no diminution in the numbers was apparent. When introduced into sterilised distilled water (which is water as chemically pure as it is possible to obtain, in which the organic matter is reduced to the merest trace) extensive multiplication also took place. (The temperature at which these samples were kept is not given.)

Again, in order to dispose of any lurking suspicion that this enormous multiplication in distilled water might be due either to a trace of organic matter introduced along with the bacteria, or to the breaking up into their constituent individuals of zoogloea-masses of the bacteria inoculated, Bolton made the following conclusive test. A minute trace of a pure culture of the Micrococcus aquatilis (similar experiments were made with similar results also with the Bacillus erythrosporus) was introduced into some sterilised distilled water; after three days extensive multiplication was found to have taken place, and a minute trace of this water was then inoculated into a fresh quantity of sterilised distilled water; after three days extensive multiplication was found to have taken place in this also; from the latter, again, a minute trace was taken and inoculated into a fresh quantity of distilled water, and so on up to seven times, and in each case the most abundant multiplication was found to occur.

Hence, in the case of these two water bacteria capacity for multiplication was shown even in the absence of almost every particle of organic matter.

Further experiments were made to ascertain how long one of the samples of distilled water was capable of affording means of multiplication to these remarkable bacteria. For this purpose, after the water had been used once for their support, it was resterilised and used again, this being repeated no less than six times in succession. No difference whatever was apparent between the multiplication of these organisms in the sample used for the first and that used for the sixth time.

In a paper by Wolffhügel and Riedel[1] experiments are recorded which were made with one individual water-organism which was inoculated into sterilised distilled water, and kept at from 30° to 35° C. The numbers rose from two on the day of inoculation to, on the first day, 80, the second day 750, the third day 1,260, the fourth day 1,600. These results, although not so striking as those of Meade Bolton's, yet also exhibit the power of certain bacteria to multiply in the absence of almost all organic matter.

Rosenberg,[2] again, introduced a series of water-organisms, which he had isolated from the river Main, into sterilised distilled water, and found that, whilst the majority of the individual varieties underwent rapid multiplication in a short time, three of the species employed quickly died off in the distilled water.

Since these results were published, it has been conclusively proved that there are some organisms which will live in the absence of *all* organic matter; amongst these the nitrifying organisms stand out prominently.[3]

[1] 'Die Vermehrung der Bacterien im Wasser,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. i. p. 460, 1886.

[2] 'Ueber die Bakterien des Mainwassers.' *Archiv für Hygiene*, 1886.

[3] 'The Nitrifying Process and its Specific Ferment,' Percy Frankland, *Phil. Trans.* 1890, vol. clxxxi. p. 107; Winogradsky, *Annales de l'Institut Pasteur*, vol. iv. 1890, p. 213, 257, 760. Vol. v. p. 92, 577; also *Archives*

In the great majority of the experiments referred to, the waters in which bacterial multiplication took place were kept in vessels to which the air had free access through cotton-wool stoppers, and although there is still deficient information on this point, we have reason to believe that the free access of air in this manner is favourable to such multiplication, and that if the waters are kept in closed bottles filled nearly to the stopper the multiplication may be much less marked. Thus a sample of Thames water was recently taken by one of us at Hampton in a Winchester quart bottle nearly filled to the stopper, and in this bottle the water was allowed to remain untouched for seven days, the average temperature to which it was exposed during this interval being about 10° C. At the end of the seven days the water exhibited 300 bacteria per 1 c.c., it was then distributed in sterile flasks plugged with cotton-wool, and these flasks were maintained at 8° C. and 19° C. in a refrigerator and incubator respectively, and on being submitted to bacteriological examination from time to time yielded the following results :—

Number of Bacteria in 1 *c.c. of Thames Water (Hampton)*
(Percy Frankland)

	Kept in flask plugged with cotton-wool	
June, 1893	8° C.	19° C.
On the 6th day	560,000	45,000
„ 12th „	166,000	30,000
„ 19th „	58,000	31,000

It is thus obvious that during the sojourn of the water in the stoppered bottle practically no multiplication can have taken place, whilst on introducing this water into flasks plugged with cotton-wool extensive multiplication ensued. This multiplication must have been very rapid and have been followed by a very

des Sciences biologiques, publiées par l'Institut impérial de Médecine expérimentale, à St Pétersbourg, 1892 ; Warington, *Chem. Soc. Journ.* 1891, 484.

rapid decline in the case of the water kept at 19° C., less rapid and followed by a much less rapid decline in the case of that kept at 8° C Thus the bacteria in the water maintained at 19° C. must have attained their maximum and fallen to 45,000 per 1 c.c. during the first six days, whilst the bacteria in the water kept at 8° C. still numbered 560,000 on the sixth day, but had fallen to 166,000 and 58,000 on the twelfth and nineteenth days respectively.

The results obtained with the Loch Katrine water on p. 230 similarly point to the comparatively restricted multiplication taking place in full stoppered bottles.

In this connection we may quote some incidental remarks of Wolffhügel and Riedel,[1] which indicate that they also observed some differences in the multiplication of bacteria in tightly stoppered bottles and in those plugged with cotton-wool respectively :—

'This diminution[2] in the number of micro-organisms in water takes place, as was proved by comparative experiments, in vessels plugged with cotton-wool as well as in those closed with india-rubber corks. On the other hand, it was almost generally found that in the vessels closed with india-rubber corks somewhat less multiplication of the bacteria took place than in those in which cotton-wool plugs were employed. The apparently more flourishing condition of the micro-organism in the latter vessels may possibly be due to the interchange of air being less restricted than is the case with the former.'

Multiplication of bacteria in carbonated waters.—This restricted multiplication, which apparently takes place when the supply of air to the water is limited, naturally leads us to a consideration of the case of those

[1] 'Die Vermehrung der Bacterien im Wasser,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. i., 1886, p. 463.

[2] Observed when samples of water were exposed to a low temperature.

waters which are either naturally or artificially charged with other gases, more especially carbonic anhydride. On this subject a number of experiments have been made by different investigators.

Thus, as regards the behaviour of micro-organisms in artificially prepared seltzer-water and carbonated waters generally, experiments have been made by Hochstetter,[1] Leone,[2] Pfuhl,[3] Sohnke[4] and Merkel,[5] the results obtained by Leone being the first which appeared. Leone found that whereas the Munich water contained on an average five bacteria per c.c., which on standing multiplied enormously, as we have already seen, in the same water through which carbonic anhydride had been passed no multiplication took place, the bacteria on the contrary steadily declining in numbers.

In these experiments Leone passed the gas through the water both under and without pressure, and the same results were obtained, showing clearly that the destruction of the bacteria present was due to the action of the gas itself, and not to the removal of the oxygen present. In some experiments by Percy Frankland[6] on the action of different gases on various micro-

[1] 'Ueber Mikroorganismen im künstlichen Selterwasser,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. ii. p. 1, 1887.

[2] Leone, *loc. cit.*

[3] 'Bakterioskopische Untersuchungen im Winter 1884–85,' *Deutsche militärärztl. Zeitschrift*, 1886, Jahrgang xv. Heft 1.

[4] 'Die Bakterienfrage in Bezug auf künstliche Mineral- und Kohlensaure Wässer,' *Zeitschrift für Mineralwasser-Fabrikation*, 1886, Jahrgang ii. No. 22 and 23.

[5] 'Ueber den Werth der bakteriologischen Untersuchungsmethode bei der Untersuchung von Trink- und Nutzwässern,' *Bericht über die 5te Versammlung der freien Vereinigung Bayrischer Vertreter der angewandten Chemie zu Nürnberg*, 1886; Berlin 1887, p. 38.

[6] 'On the Influence of Carbonic Anhydride and other Gases on the Development of Micro-organisms.' *Proc. Roy. Soc.*, vol. xlv., 1889, p. 292. See also Fränkl, 'Die Einwirkung der Kohlensäure auf die Lebensthätigkeit der Microorganismen,' *Zeitschrift für Hygiene*, vol. v., 1889, p. 332.

organisms, the different deleterious effect of carbonic acid gas on the B. pyocyaneus, Koch's cholera bacillus, and Finkler-Prior's bacillus when growing on gelatine plates was shown very clearly; the plates were poured as usual, and the vessel containing them filled with gas. The following are the results obtained with Koch's bacillus:—

Cholera Bacillus exposed in Plate-culture to Carbonic Acid Gas
(Percy Frankland)

Number of Colonies obtained from 1 *c.c. of a Sterilised Water attenuation of the Bacillus*

Air Plates	CO_2 Plates
4,183 (after 4 days)	0 [1]
4,440 (after 5 days)	0

[1] These plates were then transferred to a damp chamber filled with air, and examined after three days, but no colonies were found.

Sohnke confirmed the experiments of Leone, and obtained a marked diminution in the number of bacteria in the water into which carbonic acid gas had been introduced. This investigator also found that, whereas the seltzer-water prepared from well-water contained, although a smaller number of bacteria than was originally present in the water, yet a considerable quantity (200–6,600 per c.c.), the seltzer-water made from distilled water contained only from ten to thirty micro-organisms per c.c. Samples of the latter seltzer-water, when kept from one to nine months, contained still a few microbes, whilst three samples, which had been preserved from three to four years, were found to be entirely free from micro-organisms.

Pfuhl examined seltzer-water from two different manufactories in Altona, in both of which the ordinary Altona water-supply was used. In one case as many as 20,000 bacteria in a single c.c. were discovered, but as this was the only bottle examined from the manufactory in question, possibly some accidental contami-

nation may account for the large number found. From the other manufactory several bottles were examined, and only an average of from 80 to 100 per c.c. were found.

In Nürnberg samples from five seltzer-water manufactories were examined by Merkel. These samples were not freshly prepared, but had been stored for some time, and in each case only one bottle was tested. The results were very different, for whereas in one bottle only two organisms were found per c.c., in another bottle, purporting to be prepared from distilled water and liquid carbonic anhydride, 9,600 per c.c. were found, whilst in three other samples 355, 999 and 3,840 were respectively discovered. In the preparation of these last three samples the ordinary water-supply of the town was used, and this was found to contain originally only from four to five bacteria per c.c. Merkel explains this increase in the number found in the fact that the supply of water for preparing the seltzer water in question was not taken direct from the main, but was stored by these manufacturers in reservoirs, where doubtless it became subsequently contaminated, or, at any rate, in which multiplication took place.

Hochstetter examined seltzer-water (*a*) directly after its manufacture, (*b*) on standing for several days, (*c*) on standing for several months. Non-pathogenic organisms were also introduced, and their power of multiplication noted.

The water used for the manufacture of this seltzer-water in the first series of experiments was in some cases distilled water, and in others filtered distilled water; the samples were examined within a few hours of their manufacture, and the number of organisms found was extremely variable. The smallest number

per c.c. was seventy-three, whilst the largest figure reached was 75,000 ; whilst in some cases so many were present that they were impossible to estimate. The seltzer-water prepared from filtered distilled water contained larger numbers of bacteria than that from plain distilled water, and it would appear that in the process of filtration the water, instead of undergoing a diminution in its bacterial contents, received an increase, which was doubtless due to the filtering apparatus not being sufficiently often cleansed and renewed.

In the second series of experiments seltzer-water prepared from distilled water only was examined. The bottles were kept from one to fourteen days at a temperature of from 10° to 15° C., with the exception of one sample, which remained for one day in a warm room. This seltzer-water was found to contain in the first few hours after its preparation 118 microbes per c.c., whilst after standing for fourteen days ninety-two organisms were found. As many as 2,260 were found in the sample kept for one day in a warm room, which may be ascribed to the higher temperature at which it was preserved, and also to the possibility of a larger initial number being present.

There was a curious increase in the number of moulds observed in the samples preserved during several days. Owing to the extremely variable number of bacteria found in samples of seltzer-water from one and the same source, it is difficult to ascertain what was the exact effect of storage on their numbers, as for each determination a different bottle of seltzer-water had to be used. Thus, in the sample kept for nine days 914 bacteria were found per c.c., being many more than in the first sample only a few hours old, which contained 118 per c.c. Again, the sample one day old had sixty-three, whilst, as pointed out, the

sample fourteen days old had ninety-two. In the case of samples preserved for a long time (206 days) the bacterial contents were found to be as variable as in the case of freshly prepared samples, the difference ranging between one or two and over 100,000 ; but taking the experiments as a whole, it was ascertained that samples preserved, whether in a cellar or refrigerator, for close upon seven months contained a very large number of micro-organisms, so that in these examinations there was no evidence of a gradual falling off of the numbers, but, on the contrary, rather of an increase taking place during storage. These results contradict those of Leone and Sohnke, who, as pointed out above, found a diminution in the numbers on standing; but, as these investigators only examined a comparatively small number of samples, it is quite possible that in the individual bottles opened by them there may have been originally only a small number present.

As regards the experiments made on the powers of multiplication possessed by non-pathogenic bacteria when introduced into seltzer-water, the results varied according to the nature of the particular organism employed.

Thus the *Micrococcus aurantiacus* (see p. 501) multiplied enormously up to the thirteenth day ; on the sixteenth day a diminution was observed, and after eighteen days it was no longer demonstrable.

The *Bacillus prodigiosus* (see p. 440), even after a few days, began rapidly to decline in numbers, so much so that in some cases no trace of it could be found after between five and nine days, and the longest period in which its presence was still apparent was ten days.

Pink yeast (*Rosa Hefe*) behaved in a similar manner to the Bacillus prodigiosus.

A green fluorescent bacillus, obtained from seltzer-water, remained stationary as regards numbers up to the fourth day, after which, up to the ninth day, it declined, and after fourteen days had died off.

A yellow bacillus, also obtained from seltzer-water, multiplied extensively, and was found in large numbers even after thirty-three days; when examined after seventy-seven days it was no longer demonstrable. Hochstetter's experiments on the vitality of pathogenic bacteria in seltzer-water are given in the various tables, in the case of each organism investigated, in Chapter VIII.

Slater[1] has examined a number of different artificial aërated waters, and confirms the results of previous observers as regards the wide variations in the number of micro-organisms present, ranging from three up to 2,919 in 1 c.c. These waters were manufactured from river or deep-well water as supplied to London, also from private artesian wells, and were in some cases filtered through animal or vegetable charcoal before use, distilled and boiled water being also employed. Slater states that the bacterial purity of the original water is undoubtedly frequently effaced by the contaminations which occur in the process of manufacture. This investigator also found that if the carbonic anhydride were allowed to escape, the water being subsequently kept under sterile conditions, a rapid multiplication of the bacteria took place, indicating that this gas exercises a retarding action on the vitality of the bacteria present, thus:—A sample of aërated water contained, before loss of CO_2, 374 microbes in 1 c.c.; when examined seven days after it had lost

[1] 'Investigation of Artificial Mineral Waters,' *Journal of Pathology and Bacteriology*, vol. i., 1893, p. 468.

the CO_2 they were present in countless numbers. Some experiments were also made to ascertain if the degree of pressure employed produced any bacterial effect, but from the results obtained it would seem that it does not play any important part, the capricious variations in the bacterial contents being attributable rather to other causes.

It is obvious, therefore, that our knowledge concerning the influence of carbonic anhydride on the multiplication of the micro-organisms normally present in water is still in a far from satisfactory condition, although, taking the whole evidence into consideration, there appears to be little doubt that the multiplication of most forms is retarded, if not altogether inhibited, by this gas, whilst other forms are either capable of withstanding its destructive influence, or of actually multiplying in its presence. Fortunately our information concerning the deportment of some pathogenic bacteria in carbonated waters is more definite and less contradictory, but to this we shall refer specially in the tables in Chapter VIII.

The action of ozone on bacteria in water has been made the subject of some important experiments by Ohlmüller.[1] The ozone employed was generated from atmospheric air by means of Siemens tubes as modified by Fröhlich (*Elektrotechnische Zeitschrift*, 1891, Heft 26). Although ozone in the dry state has very little effect upon bacteria, when employed in a moist condition it acts as a very powerful bactericide; thus, in the following experiments the ozonised air was bubbled through water in which the bacteria were suspended:—

[1] 'Ueber die Einwirkung des Ozons auf Bakterien,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. viii., 1892, p. 229.

Action of Ozone on Anthrax Spores and Bacilli in Sterilised Distilled Water (Ohlmüller)

Exposure, Minutes	Volume of Ozonised Air in c.c.	Amount of Ozone yielded by 1 litre of air employed in Mgr.	Calculated amount of Ozone present in the air used Mgr.	Number of Microbes in 1 c.c. of Water	Action of Infected Water on Mice
			1. *Anthrax Spores*		
0	0	—	0	3,717,000	Died after 3 days
5	3,060	15·2	46·5	26,000	„ „ „
10	5,850	„	89·9	0	Still living after 14 days
20	11,850	„	180·1	0	„ „ „ „
40	23,550	„	357·8	0	„ „ „ „
			2. *Anthrax Bacilli*		
0	0	—	0	57,000	Died after 4 days
2	1,200	9·6	11·5	21,500	„ „ 3 „
5	3,010	„	28·8	5,000	„ „ 3 „
10	6,040	„	58·0	0	Remained living

The greater resistant power of the spore over the bacillar form is shown very distinctly, for whilst the spores only succumbed after the action of 89·9 mgr. of ozone, the bacilli were destroyed after the use of 58·0 mgr. Similar experiments made with the typhoid and cholera bacilli respectively showed that these organisms were still more sensitive, 19·5 mgr. being sufficient in the case of the former, and between 16·7 and 19·5 mgr. for the latter.

When, however, the ozone is introduced into waters containing organic matter, its action on the bacteria present is very considerably modified; thus, in the experiments in the table on p. 244 the organic matter was imparted to the water in the form of sheep-serum.

With an increasing amount of serum, therefore, the action of the ozone becomes markedly diminished, and it is evident that it is the presence of this organic matter which has retarded its influence, the ozone exercising its oxidising action first on the inanimate mate-

rial present. The same results were obtained when more or less highly polluted waters were examined.

The Action of Ozone on Anthrax Spores in Sterilised Distilled Water, with and without additions of Sheep-serum (Ohlmüller)

Exposure Minutes	Air used c.c.	Amount of Ozone yielded by 1 litre of Air Mgr.	Calculated amount of Ozone present Mgr.	Number of Microbes in 1 c.c. of Water
		1. *Without Serum*		
0	0	—	0	1,171,800
		16·5		
5	1,550	,,	25·6	201,600
10	2,750	,,	45·4	10
20	5,600	,,	92·4	0
		2. *With an addition of* 0·25 *per cent. Serum*		
0	0	—	0	1,436,400
		15·1		
5	1,450	,,	21·9	793,800
10	3,450	,,	52·1	1,008,000
20	5,300	,,	80·0	693,000
		3. *With an addition of* 0·5 *per cent. Serum*		
0	0	—	0	1,791,200
		13·1		
5	2,150	,,	28·2	1,008,000
10	5,350	,,	70·1	756,000
20	8,600	,,	112·7	1,008,000
		4. *With an addition of* 1 *per cent. Serum*		
0	0	—	0	2,520,000
		11·5		
5	2,000	,,	23·0	1,827,000
10	6,000	,,	69·0	1,890,000
20	12,350	,,	142·0	630,000

The action of peroxide of hydrogen on bacteria in water was first studied by Van Hettinga Tromp.[1] The water under investigation was thoroughly shaken up with this material, and the mixture was examined after standing for various lengths of time. An addition of 1 : 10,000, preserved for one day, was usually sufficient to sterilise a water, but the rapidity of the action of the

[1] *Waterstoffsuperoxyde-ter Desinfectie van Drinkwater.*

peroxide of hydrogen was found to depend upon the number and variety of the microbes present. Thus a water containing 19,600 microbes per c.c. was sterilised within a day by the addition of 1 : 50,000, whilst a water containing originally 34,850 organisms per c.c. required an addition of 1 : 10,000 parts of the water.

Water purposely infected with cholera organisms was rendered sterile in 5 minutes by the addition of 1 : 10,000, whilst in similar experiments with the typhoid bacillus, the latter required a whole day's exposure to an addition of 1 : 5000 before they were destroyed.

Altehoefer [1] examined the action of the peroxide on Rostock water obtained (1) from a well containing 560 bacteria per c.c., (2) from Rostock tap-water containing 180 per c.c. A sample of river-water was also employed containing 1,800 organisms per c.c. An addition of 1 : 5,000 sufficed, after being undisturbed for 24 hours, to sterilise all these waters kept at ordinary temperatures during the first 4 days, whilst all those samples to which an addition of 1 : 10,000 had been made exhibited organisms, although only a small number. On the 6th day, however, growths were obtained from the samples treated with 1 : 5,000, whilst in the second series very active multiplication of the bacteria present had taken place. Similar negative results were also obtained by this author when ordinary tap-water infected with drain-water (98 c.c. tap-water and 2 c.c. drain-water, and 199 c.c. tap-water and 1 c.c. drain-water) was treated in the proportion of 1 : 5,000 and 1 : 2,500. In order to further test the action of peroxide of hydrogen on water bacteria, an addition of 1 : 1,000 parts of the water was made. (Altehoefer

[1] 'Ueber die Desinfectionskraft von Wasserstoffsuperoxyd auf Wasser,' *Centralblatt f. Bakteriologie*, vol. viii., 1890, p. 129.

remarks that the water acquires a taste immediately after the introduction of this quantity of H_2O_2; but that if the mixture is allowed to stand for 24 hours it is not perceptible, and that this addition is perfectly harmless from a dietetic point of view.) The samples examined were tap-water containing 160 microbes per c.c., and two samples of river-water containing, respectively, 600 and 6,000 organisms per c.c.

After 7 days these various samples exhibited 3 organisms (tap-water), 2 (river-water with 600 per c.c.), and 10 organisms per c.c. (river-water containing 6,000 per c.c.). Sterile tap-water (100 c.c.) was inoculated with $\frac{1}{2}$ c.c. of drain-water, and then treated with a similar proportion of peroxide of hydrogen, and on the 6th day no organisms whatever were found.

Experiments were also made with the typhoid bacillus. For this purpose 2 c.c. of a broth culture were introduced into 98 c.c. of unsterile tap-water, and peroxide of hydrogen added in the proportion of 1 : 1,000. After 6 days an examination revealed no typhoid bacilli. Similar results were obtained when 100 c.c. of sterile tap-water, to which $\frac{1}{2}$ c.c. of sterile broth was added, was inoculated with two drops of a recent typhoid broth-culture.

Cholera bacilli inoculated into sterile and unsterile tap-water, respectively, also succumbed after 24 hours' contact with this material when present in the proportion of 1 : 1,000.

According to Altehoefer, therefore, in order to obtain sterile water by means of peroxide of hydrogen it is necessary to use this material in larger quantities than recommended by Tromp, the proportion recommended as advisable being 1 : 1,000 parts of the water. The solution of peroxide of hydrogen employed by Altehoefer contained 9·70 per cent. of this material, and

this author remarks that the strength of the solution must be carefully watched, as in samples which are no longer fresh it becomes diminished, and consequently does not act so effectually.

The authors do not, however, appear to have taken notice of the fact that ordinary commercial samples of peroxide of hydrogen usually contain free sulphuric acid, the quantity of which may have materially affected the results obtained.

These results are also of interest in connection with the possible cause of the bactericidal action of light (see p. 389).

Influence of agitation on the multiplication of bacteria. A number of experiments have been conducted in order to ascertain whether the remarkable multiplication of water bacteria to which we have devoted this chapter is in any way influenced by the circumstance of the water being at rest or in violent motion.

Notwithstanding the apparent simplicity of this investigation, the effect of agitation on the multiplication of bacteria suspended in water is still a matter of dispute, the results in the several experiments made being very contradictory. Poehl,[1] in a paper published in 1884, describes some investigations made by him on this subject. Bottles of water were attached to a centrifugal machine, and after one hour's agitation samples were submitted to plate-cultivation, and the results compared with those obtained from the same water before agitation. The most astonishing results were recorded, for it was found that a water containing originally 4,147 microbes per c.c., after being simply violently shaken with the hand for one hour, contained 728, whilst after the centrifugal agitation for the same length of time only 533

[1] *Chemische und Bakteriologische Untersuchungen betreffend die Wasserversorgung St. Petersburgs*, Petersburg, 1884, p. 24.

organisms were discoverable; again, another water having as many as 25,558 bacteria in a c.c. to start with, after one hour's centrifugal agitation was found to contain only 3,692. Urine was also tried, and the original number of 9,118 micro-organisms per c.c. was reduced, after one hour's centrifugal agitation, to 104.

Cramer,[1] on the other hand, obtained entirely negative results in similar experiments, for after shaking up a sample of water for 15 minutes, and comparing the plate-cultures with those obtained from the same water which had remained at rest, the mean of 8 experiments yielded 87 microbes per c.c. for the agitated, and 80 per c.c. for the unagitated water. Similar negative results were also obtained by Leone,[1] Miquel,[1] as well as by Tiemann and Gärtner.[1] In a paper on the effect of centrifugal agitation on the bacteria in milk, Scheurlen[2] describes some experiments which he made on their presence in water when submitted to this treatment. Anthrax-spore cultures were employed in sterile distilled water, and it was found that one hour's centrifugal agitation produced no effect either upon the numbers originally present, or on the toxic properties of the organisms, for mice on being inoculated with some of the agitated water died exhibiting typical anthrax symptoms. Scheurlen took the precaution of carefully shaking the agitated samples before examining the water, so that any bacteria which might have been precipitated during the centrifugal movement would again be distributed throughout the liquid. The different results previously obtained by Poehl, to which we have referred above, Scheurlen explains as probably due to a neglect

[1] *Untersuchung des Wassers*, Tiemann-Gärtner, 1889, p. 536.

[2] 'Ueber die Wirkung des Centrifugirens auf Bakteriensuspensionen, besonders auf die Vertheilung der Bakterien in der Milch,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. vii., 1891, p. 269.

of this precaution on the part of the former, and mentions that in one experiment in which he used tubercle bacilli, in spite of every effort being made to obtain the latter in an isolated condition, on an examination of the water after agitation in the centrifugal machine, and *without additional shaking*, very few and only isolated bacilli were found in the upper and middle layers of the water, whilst at the bottom of the vessel numerous conglomerates of bacilli often consisting of more than 100 individuals were noticed. The precise figures given are: 50 tubercle bacilli per c.c. in the original suspension, 10–15 in the upper layers, 20–25 in the middle and 1,000 and more at the bottom, after 10 minutes' centrifugal action.

Experiments were also made with other organisms, and in all cases Scheurlen found after agitation in the machine that more organisms were present in the bottom than in the upper layers of water.

This explanation of Poehl's results does not, however, appear to us as adequate, inasmuch as Poehl professes to have obtained similar reductions in the numbers of bacteria when the bottles were shaken with the hand, and not with the centrifugal machine at all.

The above represent only a few out of a large number of experiments which bear directly or indirectly on this important and interesting question, as to whether mechanical motion is favourable, unfavourable, or without effect on the multiplication of bacteria. In order, however, to indicate the contradictory results which have been obtained we cannot do better than quote the following passage from a paper by Wolffhügel and Riedel (*Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. i., 1886, p. 463), in which this subject is reviewed :—

'Alexis Horvath (*Pflüger's Archiv f. Physiologie*, 1878,

xvii. pp. 125 and 129) made some investigations in which he demonstrated that bacteria in liquid culture media multiplied when subjected to slight movements, but that when violently agitated under otherwise similar conditions no multiplication took place. From these observations he concluded that a certain degree of movement may hinder, and even entirely prevent, the multiplication of bacteria. Similar results were obtained previously by Paul Bert. C. v. Nägeli (*Theorie der Gährung*, München, 1879, p. 88) exhaustively criticised the theories and experiments of Horvath, and stated that although he did not regard his assumptions as incorrect or impossible, yet he considered it of the greatest importance that the experiments should be repeated with better culture media and at lower temperatures. E. Ch. Hansen (*Just's botanischer Jahresbericht*, 1879, p. 556) was unable to support Horvath's hypothesis without further confirmation, inasmuch as he found that Saccharomyces cerevisiæ, when cultivated in beer-wort, grew better whilst it was stirred round than when it was left at rest. J. Reinke (*Pflüger's Archiv f. Physiologie*, 1880, xxiii. p. 434) carried out some investigations on the effect of mechanical agitation upon bacteria, in which he was guided by Nägeli's criticisms. Reinke not only employed the motion of translation as did Horvath, but also the molecular motion obtained in longitudinal sound waves. These experiments confirmed Horvath's, inasmuch as it was found that molecular motion retarded the growth and multiplication of the bacteria. Reinke remarks, however, that he has no proof that it would be possible to obtain a form of mechanical movement which, by continuous application, would kill the bacteria. Buchner (*Sitzungsberichte der königl. bayer. Akademie der Wissenschaften, Mathem.-physik. Klasse*, 1880, Heft. iii., pp. 382

and 406) during his cultivations of the hay bacillus in blood employed, amongst other appliances, an apparatus for shaking them, and states that they multiplied abundantly, and did not form spores. On the other hand, Wernich (*Desinfectionslehre*, Wien-Leipzig, 1880, p. 74) states that mechanical agitation of liquid culture media (such as the bubbling through of innocuous gases, deliberate shaking of the culture vessel, stirring up of the contents, and even the ordinary constant carrying of the vessels backwards and forwards) exercised a deleterious effect upon the cultivation. In the course of his investigations on the influence of oxygen on fermentations, Hoppe-Seyler employed an apparatus which was attached to the vertical axis of a water motor, so that the liquid in the vessel flowed over the sides, and offered a large surface to the superposed air. The quiet, regular movements which were thus produced in the liquid exercised no deleterious effect upon the fermentative bacteria; for, on the contrary, they developed luxuriantly, elaborating an abundant supply of fermentation products. Tumas (*St. Petersburger medic. Wochenschrift*, 1881, No. 18) states as the result of his investigations that the most favourable condition for the development of at any rate some low organisms was not offered by the complete rest of the culture media, but, on the contrary, by their movement. The latter, however, must not be strong or violent, but moderate. Both Tumas and Hoppe-Seyler expressly state that they do not regard their results as contradicting those obtained by Horvath. Roser (*Beiträge z. Biologie niederster Organismen*, Marburg, 1881, p. 18) asserts as the result of comparative experiments that he invariably noticed a more rapid multiplication of the bacteria in those culture liquids through which air was rapidly drawn, and in which, therefore, the contents of the

vessel were kept in continuous and somewhat rapid movement, than in those left at rest.'

Behaviour of bacteria in ice.—Closely connected with the subject of bacterial multiplication, to which this chapter has been mainly devoted, is that of the behaviour of bacteria in ice, regarding which a considerable amount of information has already been obtained. Thus, some exceedingly instructive investigations on the behaviour of pathogenic and other micro-organisms in artificially prepared ice have been made by Prudden.[1] The bacteria used in these experiments were the B. prodigiosus (see p. 440), Proteus vulgaris (see p. 420), a liquefying bacillus obtained from water, Staphylococcus pyogenes aureus (see p. 498), a fluorescent bacillus and the typhoid bacillus (see p. 410). These organisms were inoculated severally into samples of sterilised water, which were then exposed by means of a freezing-machine to a temperature of 14–30° Fahrenheit for a period of 103 days. It was found that the number of B. prodigiosus originally present in the sterile water diminished from 6,300 per c.c. in 4 days to 3,000; in 37 days the numbers had fallen to 22, and at the end of 51 days none were discoverable. The Proteus vulgaris, starting with 8,300 per c.c., in 18 days was reduced to 88, and after 51 days none were discoverable. The liquefying bacillus from water, being present in such large numbers as 800,000 per c.c., in 4 days was entirely destroyed. Staphylococcus pyogenes aureus, inoculated into water from a fresh agar-agar culture, and initially present in uncountable numbers in the c.c., was still present to the number of 50,000 per c.c. after 66 days, whilst when taken from

[1] 'On Bacteria in Ice and their relations to Disease,' *New York Medical Record*, 1887, March 26 and April 2; *Centralblatt für Bakteriologie*, vol. i. p. 650, 1887.

an old and half dried up culture none were found after 7 days. The fluorescent bacillus still exhibited as many as 85,000 per c.c. after 77 days. The typhoid bacillus, which after 11 days showed 1 million per c.c. to be present, after 77 days 72,000, and after 103 days 7,000.

In these experiments the organisms were exposed to an uninterrupted low freezing temperature; but Prudden states that if the temperature is varied, and the ice allowed to thaw and then freeze again, the process is far more detrimental to the organisms. Thus, typhoid bacilli frozen for 24 hours, interrupted by 3 thawings, were reduced in this time from 40,000 at the commencement to 90, and were entirely destroyed by the end of 3 days.

Recently experiments have been made by one of us, in conjunction with Dr. Templeman of Dundee, on the effect of repeatedly freezing water containing the spores and bacilli of anthrax respectively. A mixture of spores and bacilli was obtained from an agar-agar culture of anthrax, which had been growing for 12 days at 18–20° C., and was introduced into steam-sterilised Dundee water. This infected water at the outset of the experiments yielded about 15,000 colonies to the c.c., and during a period extending over three months it was frozen by means of a mixture of ice and salt no less than twenty-nine times; on each occasion the temperature was rapidly reduced to −20° C. by means of the freezing mixture, about twenty-four hours elapsing before the whole of the ice produced in the water had again disappeared, and during the intervals between the successive freezings the infected water was kept in a dark cupboard at 9–15° C. After being frozen twenty-nine times this water yielded about 3,000 colonies to the c.c., whilst some of the same water, preserved as a control without being subjected to freezing, yielded

about 17,000 anthrax colonies per c.c. That the anthrax spores were practically unaffected by this repeated application of cold was shown by the fact that the first freezing already reduced the number of anthrax colonies to about 3,500, so that the bacilli were probably destroyed in one freezing, and the spores which had survived this first frost did not succumb either to the twenty-eight refrigerations which followed.

Very different was the behaviour of anthrax bacilli free from spores, taken from a mouse dead of anthrax, and which were similarly introduced into steam-sterilised Dundee water. This infected water yielded in the first instance about 8,000 anthrax colonies per c.c., and on examination two days subsequently, after having been once frozen, 4 out of 6 plates were entirely sterile, a fifth contained 2, and the sixth 7 anthrax colonies per c.c.; the control water, which had been kept in the dark at 10–12° C., yielded on the same day 6, 10, 135, and 236 anthrax colonies per c.c. On the fifth day the anthrax bacilli had disappeared altogether, both from the water which had now been twice frozen, as well as from the control which had been preserved throughout at 10–12° C.

As regards the behaviour of cholera bacilli in artificially frozen ice, Renk [1] has shown that after five days' uninterrupted exposure to a temperature of −0·5° and −7°C. in a freezing mixture the bacilli are destroyed, but that if the freezing was interrupted from six to seven days were necessary for their annihilation. In these experiments sterilised river Saale water was infected with cholera broth cultures. In unsterilised Saale water exposed to these low temperatures the cholera bacilli disappeared after three days, and the ordinary

[1] 'Ueber das Verhalten der Cholerabacillen im Eise,' *Fortschritte der Med.* 1893, No. 10, p. 396.

water bacteria present were reduced from 1,483,000 to 62,445 in 1 c.c. at the end of twenty-four hours, whilst after three days only 4,480 were found. Abel[1] states that, when exposed to a temperature of −20° C., the cholera bacilli may be destroyed after three days, and with certainty after eight days.

In connection with the exposure of organisms to freezing temperatures, some investigations made by Heyroth[2] are of interest, although the medium employed was gelatine and not water. The organisms were inoculated into gelatine tubes, and these were then placed for some days in a freezing mixture consisting of salt and snow. The temperature would be presumably −20° C., certainly not below. As the inoculations were made, with the exception of anthrax, from somewhat old cultivations, it is probable that in many cases spores were introduced. The gelatine tubes after being removed from the freezing-mixture were incubated at a suitable temperature, and subsequently examined to ascertain whether growth had taken place or not (see table on p. 256).

Special investigations were made with the bacilli and spores of anthrax, the former being obtained from an animal just dead of anthrax.

Anthrax spores		Anthrax bacilli	
Duration of freezing	Subsequent behaviour of culture	Duration of freezing	Subsequent behaviour of culture
1 hour . .	Growth	1 hour . .	Growth
2 hours . .	,,	2 hours . .	,,
3 ,, . .	,,	3 ,, . . .	,,
4 ,, . .	,,	3½ ,, . . .	,,
28 ,, . .	,,	—	—

[1] *Centralblatt f. Bakteriologie*, vol. xiv., 1893, p. 184.

[2] 'Ueber den Reinlichkeitszustand des natürlichen und künstlichen Eises,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. iv., 1888, p. 1.

Vitality of Bacteria in Gelatine Cultures kept in Freezing Mixture
(Heyroth)

Name of organism	Behaviour of culture after seven days' freezing	Name of organism	Behaviour of culture after ten days' freezing
Staphylococcus aureus	Growth — Less liquefaction than usual of the gelatine	Pink yeast	Growth
„ albus		Staphylococcus aureus	„
„ osteomyelitis		„ osteomyelitis	„
Streptococcus pyogenes	„	Swine fever	„
Bacillus prodigiosus	„	Rabbit septicæmia	No growth
Swine fever [1]	No growth	Bacillus pyocyaneus	Growth
„ plague [2]	Growth	Yellow sarcina	„
Calf pneumonia	No growth	Achrococcus tetragenus	„
Pigeon diphtheria	Growth	Pneumococcus (Friedländer)	„
Sporeless anthrax	No growth	Cheese spirilla (Deneke)	„
Erysipelas	Growth	Mayhöfer's bacillus	„
Finkler-Prior's bacillus	„	Coccus of septicæmia (Flügge)	„
		Miller's bacillus	„
		Bacillus of blue milk	„
		Bacillus acidi lactici	„
		Proteus vulgaris	„
		„ Zenkeri	„
		„ mirabilis	„

[1] Very similar to the bacillus of chicken cholera, but whereas the latter is pathogenic for fowls and pigeons and not for guinea-pigs, the former is almost without effect on fowls and pigeons, but acts very virulently on guinea-pigs.—*Günther*, p. 262.

[2] Rouget des porcs. Probably identical with the bacillus of mouse septicæmia.

CHAPTER VII

THE DETECTION OF PATHOGENIC BACTERIA IN WATER

So far we have been considering the presence in water and powers of growth and multiplication possessed by *ordinary water bacteria* when maintained under varying conditions of temperature, aëration, quality of water in which they were either normally present or into which they were artificially introduced, &c. We have so far, however, purposely abstained from the consideration in this respect of any bacteria known to possess pathogenic properties (excepting in the case of ice), as to this important branch of the subject we now propose directing our special attention. Before discussing the numerous experiments which have been made on the vitality of particular pathogenic bacteria when purposely introduced into various waters, it will be convenient to learn to what extent disease germs have been found *normally* present in water.

Already in 1878 Pasteur[1] found that animals into which he had injected some impure water developed symptoms of septicæmia, showing that this water must have contained amongst others certainly some pathogenic bacteria; Schuschny and Fodor[2] obtained similar results in the case of rabbits inoculated with impure

[1] *Bulletin de l'Académie de Médecine*, 1878.

[2] *Archiv f. Hygiene*, 1885, vol. iii. p. 118. (See 'Die hygienische Beurtheilung des Trinkwassers,' Hueppe, *Schilling's Journal für Gasbeleuchtung und Wasserversorgung*, 1887.)

water. Gaffky[1] succeeded, however, in not only inducing symptoms of septicæmia in rabbits by inoculating them with water taken from the highly polluted river Panke (a small stream which runs into the Spree at Berlin), but he was also able to isolate the particular bacterium from the blood and organs of these infected rabbits. It proved to be a very virulent micro-organism, for a mouse inoculated with a portion of the liver, which was teeming with bacteria, of one of these infected rabbits died in about thirty-six hours. This is the organism which is now commonly known as the bacillus of rabbit septicæmia (B. cuniculicida (see p. 429), Koch-Gaffky), and which is probably identical with the bacillus of chicken cholera (B. cholerae gallinarum, Pasteur).

Lortet and Despeignes[2] passed filtered Rhône water (the water supply of Lyon) through a Chamberland filter, and injected the slimy deposit which collected on the walls of the tubes under the skin of guinea-pigs; the latter died rapidly. They also collected some of the slime which had formed on the bottom and sides of the filtering plant during the filtration of the Rhône water, and on inoculating it into animals were able to induce virulent symptoms of septicæmia.

Rintaro Mori,[3] again, inoculated drain-water subcutaneously into thirty animals—twenty-four mice, and six guinea-pigs, the former with from three to five drops, whilst the latter received 1 to 2 c.c.'s. In the course of these investigations no less than three pathogenic bacteria were isolated and described, viz.: I. Bacillus murisepticus (Koch) (see p. 428); II. Kapseltragender

[1] 'Experimentell erzeugte Septicämie,' *Mittheilungen a. d. kaiserlichen Gesundheitsamte*, vol. i., 1881, p. 102.

[2] 'Recherches sur les Microbes pathogènes des eaux potables distribuées à la Ville de Lyon,' *Revue d'Hygiène*, vol. xii. No. 5.

[3] 'Ueber pathogene Bacterien im Canalwasser,' *Zeitschrift für Hygiene*, vol. iv., 1888, p. 47.

Canalbacillus (Rintaro Mori) (see p. 430); III. Kurzer Canalbacillus (Rintaro Mori) (see p. 429). The two latter species were new, whilst the first was the well-known bacillus of mouse septicæmia previously discovered by Koch.

Jaeger[1] states that he successfully isolated a bacillus from the river Blau, a small, highly polluted tributary of the Danube, which was pathogenic to white mice. This bacillus he calls *Proteus fluorescens* (see p. 421), and asserts that he discovered it also in the carcases of birds which had died of a mysterious disease at a small village through which the Blau flows, and where the practice was to throw such carcases into the stream as the readiest means of getting rid of them. Jaeger attributes an outbreak of jaundice-fever (Weyl's disease) which occurred in a military station situated on the stream in question to the soldiers having become infected with this bacillus whilst bathing in its waters.

Lortet[2] alleges that he was able to isolate the tetanus bacillus (see p. 423) from mud obtained from the bottom of the Dead Sea.

G. Roux[3] also states that he has found the tetanus bacillus in large numbers in the sediment of the filter-beds belonging to the waterworks supplying Lyon with the river Rhône water. Miquel has also found this bacillus in the rivers Seine and Marne.

Lortet[4] has also examined the mud taken from the bottom of the Lake of Geneva. The water in the neighbourhood of the spot from which the sample was

[1] 'Die Aetiologie des infectiösen fieberhaften Icterus,' *Zeitschrift für Hygiene*, vol. xii., 1892, p. 525.

[2] 'Microbes pathogènes des Vases de la Mer Morte,' *Lyon Méd.*, 1891, No. 33; *Centralblatt für Bakteriologie*, vol. x., 1891, p. 567.

[3] *Précis d'Analyse Microbiologique des Eaux*, Paris, 1892, p. 244.

[4] 'Die pathogenen Bakterien des tiefen Schlammes im Genfer See,' *Centralblatt für Bakteriologie*, vol. ix., 1891, p. 709.

abstracted is chemically very pure, but in spite of this the mud was found to contain pathogenic bacteria which proved fatal when introduced into animals. Lortet states that he succeeded in isolating the *Staphylococcus pyogenes aureus* (see p. 498), the tetanus bacillus, the *B. coli communis* (see p. 411), and the typhoid bacillus (see p. 410) from this muddy deposit.

Sanarelli[1] discovered an organism in the water supplying the laboratory in the University of Siena which not only proved pathogenic to cold-blooded animals, frogs, toads, eels, and other fish, but also in varying degrees to guinea-pigs, rabbits, dogs, cats, mice, hedgehogs, fowls, and pigeons. In consequence of the colour to which it gives rise when cultivated on potatoes Sanarelli has called it *Bacillus hydrophilus fuscus* (see p. 431). Water from other sources was investigated to see if this organism was present, and out of twenty-six different waters examined two were found in which it was detected.

It has recently been stated by Casado y Fernandez[2] that the tubercle bacillus (see p. 422) was found in ditch-water, and that a child contracted tuberculosis through drinking this water.

Although, perhaps, no organism has been so exhaustively studied as regards its behaviour when artificially introduced into various waters as the *B. anthracis* (see p. 416) it had, until a short time ago, never been with certainty detected as occurring in water. Additional interest and importance must, however, now be attached to the numerous researches which have been made in this direction with anthrax, as this

[1] 'Ueber einen neuen Mikroorganismus des Wassers,' *Centralblatt für Bakteriologie*, vol. ix., 1891, p. 193.

[2] 'Infeccion tuberculosa por el agua contaminada,' *Revista de Medicina y Cirugía Práctica*, 1890.

bacillus has been discovered in the mud at the bottom of a well used for drinking purposes.[1] Numerous cases of splenic fever had broken out amongst some sheep on a farm in the south of Russia, every precaution was taken to root out the disease, but healthy animals invariably developed symptoms of anthrax when they were brought back to one particular sheep-fold. At last suspicion fell on a well which was used for the animals in this fold, and a careful examination revealed beyond doubt the presence of anthrax germs in the sediment. On the closing of the well no further cases of anthrax took place. That the germs of anthrax had gained access to the well is certain, and the possibility of such contamination taking place through the drainage from soil shows how desirable is the disposal of infected carcases by cremation rather than by burial.

Thus a number of bacteria, possessing pathogenic properties of the most pronounced character, have been detected in natural waters from time to time. Several of the organisms referred to above, however, are in some respects of subsidiary interest only, as they are not pathogenic to man, whilst in no case is the propagation of any of the above diseases commonly associated with water. In fact, practically the only human diseases of the zymotic class, which are believed to be commonly propagated by water, are Asiatic cholera and typhoid fever; but the detection of the exciting causes of these diseases in water, or, for the matter of that, in any other materials, is surrounded with special difficulties, because these diseases being apparently restricted to the human species and unknown amongst the lower animals, it is impossible to test for the presence of their exciting causes by direct experiments on animals. But

[1] 'Bactéries Charbonneuses dans la Vase du Fond d'un Puits,' Diatroptoff, *Annales de l'Institut Pasteur*, vol. vii., 1893, p. 286.

it is precisely by such direct experiments on animals that nearly all the above-mentioned pathogenic bacteria have been discovered in water, the animal system serving to sift out, as it were, the few pathogenic individuals from an overwhelming majority of harmless forms with which the former must invariably be accompanied in natural waters. In the case of the microbes of typhoid and cholera then, since the animal test is impossible, recourse must be had for their detection and isolation from water to the far more laborious and less delicate method of artificial cultivation. In consequence, however, of the general enormous preponderance of the common water bacteria, the ordinary process of gelatine-plate cultivation will only, in the most exceptional cases, lead to the detection of the pathogenic forms, and special methods have had to be devised in which the growth and multiplication of the latter is favoured and stimulated, whilst the proliferation of the other bacteria present is either retarded or inhibited altogether. It is precisely in connection with such special methods for the discovery of particular forms that some of the most important advances have recently been made in the bacteriology of water.

Koch,[1] whilst engaged upon investigating the cause of cholera in India, discovered cholera bacilli in a tank which was used for drinking purposes. An epidemic of cholera had broken out in a small village in the neighbourhood of Calcutta, and the attention of the cholera commissioners was attracted to the tanks which not only supplied water for drinking purposes, but in which the natives bathed and also washed their clothes, and to which, in addition, sewage gained easy access.

[1] *Berl. klinische Wochenschrift*, 1884, Nos. 31, 32, 32a ; also ' Bericht über die Thätigkeit der Cholera-Kommission,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. iii., 1887, p. 182.

It was ascertained that the linen worn by a cholera patient had been washed in one of these tanks, and Koch was able to detect the same organism in this water which he had before isolated from the excreta and from the intestinal contents of persons who had died of cholera, and to which he gave the name of '*comma bacillus*' (see p. 399). It was first found on February 8, and was still discoverable up to the 23rd, thus showing that during at least fourteen days the cholera bacilli were able to maintain their vitality in this water.

Nicati and Rietsch [1] state that they found cholera bacilli in the water of the old harbour at Marseilles during a cholera epidemic.

During the Hamburg epidemic of 1892, cholera bacilli were on several occasions found in water by various observers; thus C. Fränkel [2] states that he was able to isolate Koch's comma bacillus from the harbour water at Duisburg. He mentions that a ship with a cholera patient on board had anchored in the harbour about five days before he received the sample of water, and that the excreta of this patient had been thrown into the adjacent water.

Loeffler [3] again detected the cholera bacilli at Demmin, in Pomerania, in a water vessel belonging to a house where a death from cholera had occurred. An examination of the well from which the water had been obtained failed to reveal the presence of cholera organisms, and it is probable, therefore, that the water had become contaminated after being brought into the house.

[1] *Rev. d'Hygiène*, May 20, 1885.

[2] 'Nachweis der Cholerabakterien im Flusswasser,' C. Fränkel, *Deutsche med. Wochenschrift*, 1892, No. 41; *Centralblatt für Bakteriologie*, vol. xii. p. 914.

[3] 'Zum Nachweis der Cholerabakterien im Wasser,' *Centralblatt für Bakteriologie*, vol. xiii. p. 380.

Lubarsch [1] also found cholera bacilli in the bilge-water of a steamer on the river Elbe.

The bacillus generally believed to be the exciting cause of typhoid fever, and known as Eberth-Gaffky's bacillus (see p. 410), has on a number of occasions been actually found in water suspected of giving rise to typhoid fever.[2]

The first investigator who discovered the typhoid bacilli in water was Moers,[3] who isolated them from a contaminated well supplying a number of people with drinking water, amongst whom many cases of typhoid fever had occurred.

This discovery was followed by a similar announcement from Michael,[4] in Dresden, who isolated the bacilli from a well-water which was suspected of being the origin of the outbreak of typhoid fever which declared itself at the end of the year 1885.

Dreyfus-Brisac and Widal [5] again detected the bacilli in polluted water obtained from a well in Ménilmontant, where typhoid fever had been prevalent for some months.

Chantemesse and Widal [6] detected typhoid bacilli three times in the river Seine water during an outbreak of typhoid fever in Paris. Thoinot [7] was also able to

[1] *Deutsche medicinische Wochenschrift*, 1892, No. 43.

[2] See article on 'Water,' by Percy Frankland, in *Thorpe's Dictionary of Applied Chemistry*, 1893, vol. iii.

[3] 'Die Brunnen der Stadt Mülheim a. Rhein vom bakteriologischen Standpunkte aus betrachtet,' *Ergänzungsh. zum Centralblatt für allgem. Gesundheitspflege*, vol. ii., 1886, p. 144.

[4] 'Typhusbacillen im Trinkwasser,' *Fortschritte der Medicin*, vol. iv., 1886, p. 353.

[5] 'Epidémie de Famille de Fièvre typhoïde,' *Gaz. hebdom.*, 1886, No. 45.

[6] *Gazette hebdomadaire de Médecine et de Chirurgie*, 1887, pp. 146–150; *Centralblatt für Bakteriologie*, vol. i., 1887, p. 682.

[7] *La Semaine médicale*, 1887, No. 14, p. 135; *Centralblatt für Bakteriologie*, vol. ii., 1887, p. 39.

isolate typhoid bacilli from the river Seine at Ivry at a distance of little more than twenty yards from the point where the water is abstracted for the supply of Paris with drinking water.

Beumer [1] was able to detect the typhoid bacillus in a well-water used for drinking purposes in a country place near Greifswald, where an outbreak of typhoid fever had occurred.

Brouardel and Chantemesse,[2] in the course of an investigation which they conducted into the causes of an epidemic of typhoid fever which prevailed at Clermont-Ferrand, claims to have discovered the typhoid baccillus in the water supplying that place as well as other places in the neighbourhood which were affected with the disease.

Henrijean [3] found typhoid bacilli in the drinking water supplying a village in Belgium during an epidemic of typhoid fever.

Kamen [4] detected typhoid bacilli in water supplying a military garrison, amongst whom typhoid fever had broken out.

Loir [5] discovered the typhoid bacillus in river Seine water which was being distributed to a portion of Paris during the summer of 1887, in consequence of the scarcity of the Vanne water, which yields the supply under ordinary circumstances.

[1] 'Zur Aetiologie des Typhus abdominalis,' *Deutsche medicinische Wochenschrift*, 1887, No. 28.

[2] 'Enquête sur les Causes de l'Epidémie de Fièvre typhoïde qui a régné à Clermont-Ferrand,' *Annales d'Hygiène publique et de Médecine légale*, vol. xvii., 1887, pp. 385–403; also *Revue d'Hygiène*, vol. ix. p. 368.

[3] 'Contribution à l'Etude du Rôle étiologique de l'Eau potable dans les Epidémies de Typhus,' *Annales de Micrographie*, vol. ii. p. 401.

[4] 'Zum Nachweise der Typhusbacillen im Trinkwasser,' *Centralblatt für Bakteriologie*, vol. xi. p. 32.

[5] 'Recherche du Bacille typhique dans les Eaux d'Alimentation de la Ville de Paris,' *Annales de l'Institut Pasteur*, vol. i. p. 488.

Péré[1] states that he was able to isolate the typhoid bacillus from drinking water in Algiers, where typhoid fever is endemic, making its appearance every year in the months of August, September, and October.

Vincent,[2] confirming the researches of previous investigators,[3] again found the typhoid bacillus in the Seine water being supplied to Paris during the summer of 1890.

Martin,[4] in a paper dealing with an outbreak of typhoid fever in Bordeaux, states that the typhoid bacillus was found by Ponchet in the public water supply.

Fodor,[5] in a paper read before the International Hygienic Congress held in London in 1891, describes an outbreak of typhoid fever at Budapest, in which he succeeded in detecting the typhoid bacillus five times in the public water-supply. It was afterwards ascertained that the waste water from a laundry attached to the hospital gained direct access to the principal water main in the town.

Kowalski[6] states that in 2,000 samples of water which he examined bacteriologically he was only able in five to detect typhoid bacilli.

Finkelnburg[7] describes the detection of typhoid

[1] 'Contribution à l'Etude des Eaux d'Alger,' *Annales de l'Institut Pasteur*, vol. v. p. 79.

[2] 'Présence du Bacille typhique dans l'Eau de Seine pendant le mois de Juillet, 1890,' *Annales de l'Institut Pasteur*, vol. iv. p. 772.

[3] Loir, see above; Thoinot, *Bulletin de l'Académie de Médecine*, 1887.

[4] 'Présence du Bacille typhique dans les Eaux d'Alimentation de la Ville de Bordeaux,' *Revue sanit. de la Province*, 1891, No. 181, p. 93; *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 413.

[5] 'Die Beziehungen des Typhus zum Trinkwasser,' *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 121.

[6] 'Ueber bakteriologische Wasseruntersuchungen, *Wiener klinische Wochenschrift*, 1888; *Centralblatt für Bakteriologie*, vol. iv., 1888, p. 467.

[7] 'Ueber einen Befund von Typhusbacillen im Brunnenwasser,' *Centralblatt für Bakteriologie*, vol. ix., 1891, p. 301.

bacilli in a well-water which was situated in close proximity to a privy. This author mentions that he was only able to discover them by examining the deposit of the water by means of an apparatus specially constructed by him for the investigation of the sediment left by water. The author suggests this as a useful method for detecting other pathogenic organisms in water.

We will refer the reader to the account, which will be found later, of the special methods which have been devised for the detection of typhoid bacilli in water in the presence of other organisms. It is possible that in some of the earlier investigations which we have recorded, although the typhoid bacillus was very possibly present in the water, yet its actual detection, although alleged to have been accomplished, remains doubtful, as the methods employed were not so accurate as those more recently used. In this connection we would also refer the reader to the elaborate papers of Cassedebat [2] and Dunbar,[3] which discuss in detail the endeavours which have been made to differentiate between the typhoid bacillus and other forms closely resembling it which are also found in water.

Detection of Typhoid Bacillus in Water

We have already drawn attention to the fact that doubt attaches to the identification of the typhoid bacillus in waters recorded by some of the earlier investigators to whose work we have had occasion to refer above. This uncertainty is due to the almost constant presence with the typhoid bacillus in water of other organisms so closely resembling it, that their

[2] *Annales de l'Institut Pasteur*, vol. iv., 1890, p. 625.
[3] *Zeitschrift f. Hygiene*, vol. xii., 1892, p. 485.

differentiation is a matter of extreme difficulty. The particular microbe which has given rise to so much confusion is the so-called *B. coli communis* (see p. 411). This organism was described by Escherich, and is found regularly in the intestinal tract as well as in human fæces, also in the excreta from animals, and is regarded as identical with the Bacillus neapolitanus of Emmerich and the Fæces-bacillus described by Weisser. In all cases, therefore, where water is supposed to have been infected by the fæces of typhoid patients the B. coli communis may be expected also to be present. This organism is in fact frequently found in great numbers in polluted streams, as well as in well-waters into which the drainage from dung-heaps has penetrated, whilst in pure waters it is but rarely met with. Therefore, in order to ascertain definitely whether the typhoid bacillus is present in any given water, care must be taken that the B. coli communis is not mistaken for the former, and to guard against this some method must be adopted which will, whilst revealing the presence of the typhoid bacillus, effectually eliminate or separate out its constant attendant, the B. coli communis. Unfortunately this is a matter of extreme difficulty, for the vitality of the B. coli communis in water is superior to that exhibited by the typhoid bacillus; moreover, in all attempts which have so far been made to suppress the vitality of other organisms and yet permit the development of the latter, the B. coli communis has shown itself to be possessed of greater powers of resistance than the typhoid bacillus itself. Hence, although the addition of various chemical substances may effectually destroy or retard the growth of other organisms, yet the B. coli communis survives and remains present along with the typhoid bacillus; indeed, in many cases it has been proved that such additions have *destroyed* the typhoid bacillus and left the

B. coli communis alone master of the field. It is true that, growing in artificial cultures side by side, there are certain differences which individualise these organisms, for the B. coli communis grows more luxuriantly in the various culture media employed than does the typhoid bacillus, but yet on gelatine-plates there are always colonies to be found which in every respect resemble the latter, whilst even on potatoes, which used to be considered an important test, the B. coli communis may and does exhibit under certain conditions growths which are undistinguishable from those produced by the typhoid bacillus. In two media however, according to Dunbar,[1] a marked difference is found in the behaviour of these two organisms. Whilst in sterile milk the typhoid bacillus renders the liquid slightly acid and *never* brings about its coagulation, the B. coli communis at the temperature of the body coagulates the milk in from twenty-four to forty-eight hours and at the same time renders it strongly acid. Again, when grown in sterile fluid meat-extract the B. coli communis at the above temperature produces in the course of from three to twelve hours a quantity of gas (consisting of hydrogen and carbonic anhydride), whilst no formation of gas has ever been observed in the case of the typhoid bacillus.

This distinction has been shown by one of us to be available in an extremely simple form for the differentiation of the two organisms; thus, on inoculating them respectively into test-tubes of melted gelatine-peptone (the *ordinary* mixture, see p. 9), then allowing the latter to solidify, and maintaining them at the ordinary

[1] 'Ueber den Typhusbacillus und den Bacillus Coli communis,' *Zeitschrift für Hygiene*, 1892, p. 491. See also *State Board of Health Massachusetts Report*, 1891, p. 637, 'The Differentiation of the Bacillus of Typhoid Fever,' by George N. Fuller.

temperature (18–20° C.), the tubes containing the B. coli communis invariably exhibit after 24 to 48 hours numerous conspicuous gas-bubbles distributed through the solid medium, whilst no such bubbles make their appearance in the tubes containing the typhoid bacilli. The test possibly depends upon the meat-extract containing sufficient dextrose (derived from the post-mortem transformation of the glycogen in the blood) for a visible fermentation by the B. coli communis to take place. The bubbles of gas are certainly independent of any ingredients present in either the gelatine or in the peptone, for we have found them to form in agar-agar-peptone (of the composition given on p. 16), and also in meat-extract-gelatine to which no peptone had been added. The great convenience of the test depends upon its involving only the use of a medium which must invariably at all times be at hand in every bacteriological laboratory, and also in its dispensing with the use of an incubating temperature.

From the above sketch it will be seen how many difficulties attend the successful identification of the typhoid bacillus in the presence of its constant companion, the B. coli communis, but as many of the methods which have been devised for its isolation are not only extremely ingenious, but also of great service in eliminating other organisms, a brief account is here given of the more important of these.

Uffelmann's Method.[1]—This method is based upon the idea that few micro-organisms can flourish in as acid a culture medium as the typhoid bacillus. Thus, for the demonstration of the latter in the presence of other microbes, Uffelmann uses gelatine to which a definite quantity of citric acid (eight drops of a 5 per cent. citric acid solution to 10 c.c. of gelatine) and methyl

[1] *Berliner klinische Wochenschrift*, 1891, No. 35, p. 857.

violet have been added. The typhoid colonies assume a blue colour, which becomes more intense with their age and is finally of a much deeper tint than the surrounding gelatine.

By means of this gelatine Uffelmann states that he was able to so far eliminate other organisms that a water, from which by using the ordinary gelatine 12,500 colonies were obtained, when tested by this special process only exhibited nineteen. From another sample, instead of 180, only eleven colonies appeared on the plate, and out of these Uffelmann states that six were those of the typhoid bacillus, being distinguished by the characteristic blue colour which they exhibited. Dunbar, however, has shown that this gelatine is very prejudicial to the development of the typhoid bacillus, in many cases no growth whatever appearing on the plates, whilst when the B. coli communis was experimented with it was found that they appeared abundantly, presenting the same blue colour as the typhoid colonies, although somewhat retarded in their growth. Dunbar further ascertained that the amount of citric acid prescribed by Uffelmann was altogether in excess of that which the typhoid bacilli were capable of standing, for their growth was already retarded by the addition of four drops, and that with six their development was often completely stopped, whilst the B. coli communis grew in the presence of seven to eight drops of this acid. Moreover this acid methyl-violet-gelatine, whilst preventing the growth of the typhoid bacillus, was often in Dunbar's experiments liquified by foreign organisms when very polluted waters were examined.

Holz's Method.[1]—Assuming that the growth of the typhoid bacillus upon potatoes presents quite distinct

[1] 'Untersuchungen über den Nachweis der Typhusbacillen,' *Zeitschrift für Hygiene*, vol. viii., 1890, p. 143.

features from those exhibited by other organisms, Holz prepared a culture material from potatoes, which, whilst resembling in all important features the conditions afforded by the potato, had the advantage of being transparent. (A description of the mode of preparing this culture medium will be found on p. 22.) To effect the destruction of other organisms Holz added 0·05 per cent. of carbolic acid. But as in the case of Uffelmann's medium, so with this, the B. coli communis is not eliminated, and remains side by side with the typhoid bacillus, and cannot be distinguished from it.

Parietti's Method.[1]—This method consists in adding carbolic acid and hydrochloric acid in certain proportions to neutral bouillon, in the hope of thus eliminating other organisms without destroying the typhoid bacillus; but inasmuch as the B. coli communis is able to withstand larger doses of both carbolic acid and hydrochloric acid than the typhoid bacillus, this method cannot be relied upon any more than those previously described. As this method, however, is very convenient for sifting out the typhoid and coli from a large number of other organisms, it is given in detail.

A series of test tubes containing 10 c.c. of neutral bouillon receive from 3–6–9 drops (30 drops = 1 c.c.) of the following solution :—

5 g. Carbolic acid
4 g. Pure hydrochloric acid
100 g. Distilled water

These tubes are then placed in the incubator and kept at 37° C. for twenty-four hours, in order to destroy any organisms which may have gained access during the addition of the solution. To the sterile tubes 1 to 10

[1] 'Metodo di ricerca del Bacillo del tifo nelle acque potabili,' *Rivista d' Igiene e Sanità publica*, 1890.

drops of the water under investigation are added, and after being shaken and the contents thoroughly mixed the tubes are again placed in the incubator. If after twenty-four hours' incubation any of the tubes appear turbid, they should be submitted to ordinary plate-cultivation, and the resulting colonies carefully examined for the characters which they exhibit with a view to their identification. In using this method for the detection of typhoid bacilli in water, it has, however, been found by one of us that the above incubation must be prolonged for forty-eight or even seventy-two hours when only few typhoid bacilli are present.

A further distinction which serves for the differentiation between the coli and typhoid bacilli is the so-called *indol-reaction*. This is best applied in the following manner, as recommended by Kitasato :[1]—

To 10 c.c. of the culture in ordinary alkaline peptone-broth (see p. 25) of the organism under examination, and which has been growing for twenty-four hours in the incubator, add 1 c.c. of a solution of potassium or sodium nitrite (containing ·02 gram in 100 c.c.), and then a few drops of concentrated sulphuric acid. *If indol is present, a rose to deep-red coloration is produced*, depending on the interaction of nitrous acid with indol to form nitrosoindol nitrate, which is of red colour. On applying this test to the B. coli communis an indol-reaction is obtained, whilst the typhoid bacillus gives a negative result.

Vincent's Method.[2]—In this method, which is very similar to Parietti's above, the attempt is made to

[1] *Zeitsch. f. Hygiene*, vol. vii., 1889, p. 518. Through a very serious printer's error in this paper, potassium nitr*ate* is repeatedly described as being used instead of nitr*ite*. Kitasato corrects this mistake in *ibid.* vol. viii., 1890, p. 61.

[2] *Comptes rendus hebdomadaires des Séances de la Société de Biologie*, 1890, No. 5.

destroy the organisms other than typhoid in the water itself, before proceeding to plate-cultivation. For this purpose the water to be examined is introduced into a test-tube containing sterile peptone-bouillon and five drops of a 5 per cent. solution of phenol. The water with this phenol-bouillon is then preserved at 42° C. Most of the water-bacteria are thus removed, but Vincent himself acknowledges that he was not able by this means to eliminate the B. coli communis.

Chantemesse and Widal's Method.[1]—This method consists in using for the plate-cultivation of the water under examination a gelatine-peptone medium containing ·25 per cent. of phenol. Holz has shown, however, and his results have been confirmed by Dunbar, that these authors used a percentage of carbolic acid, which altogether prevents the growth of the typhoid bacillus; and Dunbar has further shown that small additions of phenol, by impeding the growth of the colonies of B. coli, cause the latter to present even stronger resemblances than usual to the colonies of the typhoid bacillus. Dunbar has also made experiments which confirm Vincent's observations, that the B. coli communis is able to withstand a larger addition of phenol than the typhoid bacillus, so that even if a smaller amount of phenol were to be used than that recommended above by Chantemesse and Widal in their paper, no advantage would be gained, as, along with the typhoid bacillus, the B. coli communis must, if present, also invariably make its appearance.

Dunbar has further pointed out that the addition of $\frac{1}{10}$ c.c. of a 5 per cent. solution of phenol to 10 c.c. of gelatine—*i.e.* a gelatine containing 0·05 per cent. phenol, exercises a marked effect in reducing the liquefaction of the gelatine by foreign organisms, whilst this addition

[1] *Gazette des Hôpitaux*, 1887, p. 202.

has hardly any effect upon the B. coli communis, which develop almost as freely as on the control plate. Thus, in the investigation of samples of water for typhoid bacilli in which the determination of the number of the latter is not desired, and in which there is a probability of many liquefying organisms being present, such an addition may prove of much service, and may therefore be recommended, but it will not, of course, separate the typhoid from the B. coli communis.

Thoinot's Method.[1]—This consists in adding 0·25 gramme of pure phenol to 100 c.c. of the sample of water to be investigated. But an addition of 0·116 per cent. of carbolic acid greatly interferes with the growth of the typhoid bacillus, whilst it will not develop at all in the presence of 0·144 per cent. (Dunbar, *loc. cit.*, p. 491.)

It will thus be seen that there is at present no method whatever of isolating the typhoid bacillus from water which does not at the same time also isolate the B. coli communis, if the latter is present, as it almost invariably will be, for all circumstances hitherto examined which favour the growth of the typhoid bacillus relatively to that of other bacteria are equally or even more conducive to the growth of the B. coli communis, and no agency is available for the destruction of the B. coli communis which does not also destroy the vitality of the typhoid bacillus. By the judicious application, however, of some of the above methods there can be no doubt that the discovery of the typhoid bacillus is greatly facilitated.

Thus, the water should be preliminarily purified from most bacteria by culture in phenol-broth (preferably as in Parietti's method). On subsequent plate-cultivation the colonies obtained will be limited to those of B. coli communis, the typhoid bacillus, and possibly

[1] *Gazette des Hôpitaux*, 1887, p. 348.

those of a few other forms; any colonies at all resembling those of the typhoid bacillus must then be further examined—(1) *microscopically;* (2) *by cultivation on potatoes;* (3) *by inoculation into melted gelatine-tubes for the gas-bubble test;* (4) *by cultivation in milk for coagulation; and* (5) *by cultivation in broth for the indol-reaction,* before a reasonably safe diagnosis of typhoid bacilli can be made (see note p. 285.)

A description of the B. coli communis, together with some of the principal organisms which closely resemble the typhoid bacillus, will be found in the tables at the end of the book (see pp. 410–415).

Detection of Koch's Comma Bacillus in Water

As in the case of the typhoid bacillus, so with that of cholera, much difficulty is experienced in its recognition amongst a number of other bacteria. To facilitate its identification Schottelius[1] recommended the following method:—A small quantity of the choleraic dejecta is mixed with twice its quantity of slightly alkaline[2] sterile bouillon, and is then preserved at a temperature of from 30°–40° C. for twelve hours. At the end of this time an extensive multiplication of the cholera bacillus takes place *on the surface* of the liquid, a small quantity of the pellicle often exhibiting under the microscope an almost pure cultivation of the cholera bacteria. If the cultivation remains standing for two or three days, on the other hand, only very few, or perhaps no cholera bacilli will be found, the latter having been outnumbered by the other organisms present. This method may of course be applied in the case of waters suspected of harbouring the cholera bacillus.

[1] *Deutsche med. Wochenschrift*, 1885, No. 14.

[2] It is of the greatest importance that all the media employed in the culture of the cholera bacilli should be very distinctly alkaline in reaction (see p. 23).

Vestea [1] states that he was able to demonstrate by this method the presence of the cholera bacilli in dejecta suspected of being choleraic in character, although microscopic examination had failed to reveal their presence. Already at the end of fifteen hours a considerable number of the comma-shaped bacilli were found on the surface and at the edge of the liquid.

Loeffler [2] recommends the use of larger quantities of water when search is being made for the cholera organism. For this purpose, to 200 c.c. of the water should be added 10 c.c. of alkaline broth-peptone, and the mixture placed in the incubator for twenty-four hours.

Weibel,[3] who has made a special study of spirillar forms generally, draws attention to the property which many spirilla possess of growing in very diluted culture media, in which they are better able to hold their own than the other organisms present, and they may thus be more readily identified than when placed in more highly nutritive media. Thus, if some material containing a large number of vibrios, *e.g.* sewer-mud, is inoculated into ordinary broth, the other organisms present multiply so extensively that the spirilla are almost entirely crowded out. If, on the other hand, the broth be diluted with forty to fifty parts of sterile water, the spirilla remain in the majority, or at any rate gain the upper hand in the course of a few days. Weibel found by this method the *Vibrio coli* in the mucous flakes of diarrhœic fæces, which never appears on gelatine-plates, and in ordinary broth is at once crowded out by the *B. coli communis*. Weibel recommends this method for the examination of waters for spirillar forms.

[1] *Centralblatt für Bakteriologie*, vol. iii., 1888, p. 320.
[2] *Ibid.*, vol. xiii., 1893, p. 384.
[3] *Ibid.*, vol. iv., 1888, p. 294.

It should be mentioned that Dunham [1] found as far back as 1887 that the cholera bacilli multiplied very rapidly in broth containing 1 per cent. of peptone, to which 0·5 per cent. of common salt was added. Koch [2] mentions that no advantage appears to have been taken of this fact in the methods employed for the detection of the cholera bacillus in dejecta, until Dunbar brought it into practical use during the epidemic of cholera in Hamburg in 1892. Since that time the following method has been employed by Koch and others for the identification of cholera bacilli in water :—

100 c.c. of the water under examination receives 1 per cent. of peptone and 1 per cent. of common salt, after which the mixture is placed in the incubator and kept at 37° C. After intervals of ten, fifteen, and twenty hours agar-plates are poured, whilst a careful microscopic examination is also made of the mixture. Any colonies which appear on the agar-plates and resemble those of the cholera bacillus are examined microscopically, and whenever comma-shaped forms are found they are inoculated into fresh tubes so as to enable them to be further tested by means of the indol-reaction, and also by animal inoculations. Koch states that by using the above method he was able to demonstrate the presence of cholera bacilli in the river Elbe at Hamburg, in a well at Altona, in the river Saal, and in other places.

As is well known, waters of very various origin frequently contain comma-shaped bacteria, which, like the cholera bacillus, also collect in the upper layers of the liquid, so that every care must be exercised to

[1] 'Zur chemischen Reaction der Cholerabacterien,' *Zeitschrift f. Hygiene*, vol. ii., 1887, p. 337.

[2] 'Der augenblickliche Stand der Choleradiagnose,' *Zeitschrift f. Hygiene*, vol. xiv., 1893, pp. 326 and 336.

exclude the possibility of such forms being confounded with the cholera bacillus. Koch states that nearly a dozen such spirillar forms have been isolated in his laboratory from various waters, but that *the absence of the indol-reaction* as well as *the absence of any pathogenic effects on guinea-pigs* sufficiently distinguished them from the cholera bacillus. In the tables at the end of the book, on pp. 399–408, will be found a description of some of the comma-shaped bacteria isolated by different observers from waters.

Arens[1] has devised a method by means of which he says that cholera bacilli, even when present in very small numbers (two in 5 c.c. of water), may be with certainty detected. This consists in first rendering the water distinctly alkaline by the addition of 1–1·6 c.c. of a 10 per cent. solution of caustic potash to 200 c.c. of the water under examination, so that the latter contains ·05–·08 per cent. of KOH. This alkalised water then receives pancreas-bouillon in the proportion of one to nine parts of the water. The pancreas-bouillon is composed of broth obtained from the pancreas, to which Witte's peptone is added, and the whole neutralised with carbonate of soda until a highly diluted portion yields a faint red colour with rosolic acid. The treated samples of water are then incubated as in Schottelius' method, and the cholera bacilli are found on the surface of the liquid and may be easily isolated subsequently by means of plate-cultures.

Sanarelli,[2] in his investigations on the presence of spirillar forms in the rivers Seine and Marne and in drain water, adopted the following method for their isolation.

[1] 'Ueber den Nachweis weniger Cholerakeime in grösseren Mengen Trinkwassers,' *Münchener med. Wochenschrift*, 1893, No. 10.

[2] 'Les vibrions des eaux et l'Etiologie du Choléra,' *Annales de l'Institut Pasteur*, vol. vii., 1893, p. 693.

To every 100 c.c. of the water under examination the following addition of nutritive material is made, consisting of:

Gelatine	2	grms.
Dry peptone	1	„
Sodium chloride	1	„
Potassium nitrate	0·10	„

Large flasks are employed, so that as extensive a surface as possible of the treated water is exposed to the air. After being preserved for twelve hours at 37° C., a thin pellicle forms on the surface, in which, under the microscope, spirillar forms are easily recognisable. According to Sanarelli, spirilla grow so rapidly when thus treated, that if only a few are originally contained in the water, this short time is sufficient to reveal their presence. For their subsequent isolation it is only necessary to take a small piece of the pellicle, and, after mixing it with a little sterile water, to pour gelatine-plates from the dilution. This author states that he has found the presence of a large quantity of albuminoids very unfavourable to the development of spirillar forms; for this reason, in the preparation of nutritive agar-agar for their subsequent cultivation, he uses, instead of meat extract, ordinary water. In this manner an exceptionally transparent culture-material is procured, which is also especially fitted for the growth of these forms at 37° C.

Thus the crucial tests now recommended by Koch for the differentiation of the cholera bacilli from allied forms with which they are liable to be confounded are:—

1. The *positive indol-reaction.*

2. The *positive pathogenic effects on guinea-pigs*, which are yielded by the cholera bacilli, but apparently not by the allied forms as far as these have been yet examined.

It is necessary, therefore, that we should enter a little more into detail regarding these two tests, which acquire such a high importance in this connection.

The *indol-reaction*, as already described (see p. 273) in connection with the diagnosis of the typhoid bacilli and the B. coli communis, depends upon the interaction of nitrous acid with indol, resulting in the production of a red colour. The formation of indol in a bacterial culture can thus be readily ascertained by generating nitrous acid in the latter by the addition of a small quantity of a nitrite and some sulphuric acid. In the case of the ordinary cultures of the cholera bacilli, however, the nitrite is already present, having been formed by the reduction of nitrate which is almost invariably contained in the peptone used, so that the addition of sulphuric acid is alone necessary for the exhibition of the indol-reaction. The production of this red colour on the simple addition of sulphuric acid to broth cultures of cholera bacilli was originally discovered by Bujwid [1] and Dunham [2] before its cause was understood, and was named by them the *cholera-red-reaction*. In order that this reaction may be obtained with certainty, Koch points out that several precautions must be strictly observed. It is of the greatest importance that suitable peptone should be employed; this probably depends upon the fact that some samples of this material contain an insufficient or an excessive amount of nitrate respectively.[3] Secondly, the sulphuric acid employed must be absolutely free from nitrous acid; Thirdly, the test must only be applied to *pure* cultures of the spirilla, as otherwise the production of the

[1] 'Eine chemische Reaction für die Cholerabacterien,' Bujwid, *Zeitsch. f. Hygiene*, vol. ii., 1887, p. 52.

[2] 'Zur chemischen Reaction der Cholerabacterien,' Dunham, *ibid.*, p. 337.

[3] Bleisch, *Zeitsch. f. Hygiene*, vol. xiv.

indol-reaction may have been caused through other bacteria having furnished either the indol or the nitrous acid, or both. The reaction being more pronounced and more uniform in cultures in peptone-solution (1 per cent. peptone, ·5 per cent. sodium chloride) than in peptone-broth, only the former medium should be employed for the purpose.

Another spirillum which gives the cholera-red-reaction is the *Vibrio Metschnikovi* (not hitherto found in water), which also in many other respects closely resembles the cholera bacillus of Koch; it is, however, sharply distinguishable from the latter by its powerfully pathogenic properties (producing a virulent septicæmia) when subcutaneously inoculated even in the smallest quantities into guinea-pigs, and more especially pigeons.[1]

Neisser[2] found a vibrio (Vibrio Berolinensis, see p. 400) in Berlin tap-water which in every important particular resembles Koch's cholera bacillus, the only difference which could be detected being slight variations in the appearance of the colonies on gelatine-plates.

The confirmation of cholera bacilli by *animal experiment* is best effected, according to Pfeiffer,[3] by taking a full needle-loop (about ·0015 grm.) of the surface-growth on an agar-agar culture, distributing this in 1 c.c. of sterile broth, and then injecting the latter into the peritoneal cavity of a guinea-pig. The above quantity of material should be a fatal dose for an animal of

[1] For further particulars see Günther's *Bakteriologie*, Leipzig, 1893.

[2] 'Ueber einen neuen Wasser-Vibrio, der die Nitrosoindol Reaction liefert,' *Archiv für Hygiene*, 1893, p. 194.

[3] *Zeitsch. f. Hygiene*, vols. xi. and xiv. See also Sabolotny's paper in the *Centralblatt f. Bakteriologie*, vol. xv. 1894, p. 150, in which he shows that the marmot can be infected by small quantities of broth-cultures *subcutaneously* introduced. For further particulars see Remarks in Comma-Spirillum Table, p. 399.

300–350 grms. in weight, but for a larger animal a larger dose must be employed. The injection is followed by a rapid fall in temperature, ultimately resulting in death. According to Koch, the cholera bacilli are the only spirilla which have yet been discovered in the course of cholera investigations which give anything even approaching to these symptoms when the above quantity is employed.

Sanarelli (*loc. cit.*), however, recently isolated no less than thirty-two vibrios from water, morphologically distinct from each other, all of which gave the indol-reaction and some the cholera-red-reaction, four of which not only exhibited both these reactions, but were extremely pathogenic to animals, producing symptoms undistinguishable from those considered typical of cholera infection. Sanarelli is therefore of opinion that many varieties of vibrios may exist in water, morphologically distinct from the cholera vibrio, but capable of producing a disease in man and animals in its morbid aspects identical with cholera, and that the assumption that this disease is produced by only one particular variety of vibrio, as hitherto held, must be abandoned.[1]

Detection of Anthrax Spores in Water

A method has been devised by one of us[2] suitable for the detection of anthrax spores when present along with other micro-organisms in water. A large proportion of the organisms present in water, and more especially those causing liquefaction of the gelatine, are very sensitive to a temperature even considerably below that of boil-

[1] In this connection see also a more recent paper by Dunbar, 'Versuche zum Nachweis von Cholera vibrionen in Fluszwasser,' *Arbeiten a.d. Kaiserl. Gesundheitsamte*, vol. ix. 1894, p. 379.

[2] 'Experiments on the Vitality and Virulence of Sporiferous Anthrax in Potable Waters,' Percy Frankland, *Proc. Roy. Soc.*, 1893, p. 192.

ing water, whilst the spores of anthrax in their normal state will withstand such temperatures for a considerable length of time. In order to turn these properties to practical account, portions (1 c.c. or 3 c.c.) of the water supposed to contain anthrax spores are mixed with a little sterile broth (1 c.c.), and heated for periods of two or five minutes to 50° C., to 70° C., or to 90° C., after which treatment they are submitted to ordinary plate cultivation. As an illustration of the manner in which this method works the following example may be cited:—Thames water, purposely infected with anthrax spores, and containing upwards of 100,000 water bacteria in 1 c.c., had this number reduced after heating as above for five minutes to 50° C. to from thirty-five to thirty-nine per c.c., amongst which several of the colonies on the plate were recognisable as those of anthrax. Again, on the same day, other portions of the same water were heated to 70° C. for two minutes, after which only from ten to thirty colonies per c.c. made their appearance, amongst which from four to ten were recognisable as anthrax. Other portions of the same water were heated on the same day to 90° C. for two minutes, with the result that only from seven to ten colonies per c.c. appeared, of which from three to six were found to be anthrax.

By this simple method the water is deprived of its rapidly liquefying forms, which so quickly render the gelatine-plate fluid and necessitate its being thrown away before the more slowly developing anthrax colonies have been able to make their appearance. Such a water, swarming originally with water bacteria, may thus be easily examined, and the anthrax colonies identified by gelatine-plate cultures.

The longer the bacteriological examination of water is practised the more evident does it become that in

searching for pathogenic organisms special methods must be devised and adopted according to the nature of the particular microbe of which we are in quest, and that only under the most exceptional circumstances is there any possibility of such pathogenic bacteria being found in the course of ordinary plate cultivations made with natural waters, the colonies of the common water-bacteria almost invariably so predominating as to exclude all others present only in small numbers. Such special methods have, as pointed out above, already been employed, more especially for typhoid and cholera bacilli, as well as for anthrax spores; in all cases these methods should be so arranged as to permit of the examination of larger volumes of water, as in this manner the chance of discovery is correspondingly increased.

NOTE.—In examining water for the typhoid bacillus it is advisable to pass a considerable volume, 250 c.c. or upwards, through a sterile porcelain or infusorial earth filter (see p. 172), and then to transfer the deposit on the surface of the cylinder by means of a sterile brush into a small quantity of sterile water; the latter, which then contains the bacteria from the large original volume of water, should be treated by phenol-broth culture or one of the other methods of typhoid detection. To assist the diagnosis of the typhoid bacillus in the presence of the B. coli communis, Schild (*Zeitschrift f. Hygiene*, vol. xvi. 1894, p. 373) recommends the use of broth to which '*formalin*' has been added. Formalin may be procured from Schering, in Berlin, and consists of a concentrated (40 per cent.) aqueous solution of formaldehyde. It should be added by means of a sterile pipette to ordinary neutral broth, of course *after* the sterilisation of the latter, as heating volatilizes the formaldehyde. The proportion recommended by Schild is 1 : 7000, and he states that, whereas the B. coli communis will flourish in this formalin-broth, rendering it turbid in from 8–24 hours, the typhoid bacillus refuses to grow and the liquid remains clear. This formalin-broth must only be used when freshly prepared, as the formaldehyde volatilizes on being kept. Schild states that in adding the formalin to the sterile broth he has only rarely been troubled with contaminations, and then they were traced to moulds which were easily recognisable.

CHAPTER VIII

THE VITALITY OF PARTICULAR PATHOGENIC BACTERIA IN DIFFERENT WATERS

The difficulties which, in the last chapter, we have seen attend what may be called the analytical method of investigation into the fate of pathogenic bacteria gaining access to natural waters, early led to supplementary researches by what may be called the synthetic method, in which the pathogenic organisms were purposely introduced on an experimental scale into different kinds of water, which were then kept under observation and examined from time to time for the bacteria in question.

Although at first sight it might appear an easy task to thus synthetically determine the vitality of organisms in water, requiring only their introduction into various waters and the subsequent estimation of their numbers at suitable intervals of time, as a matter of fact, however, the number of problems requiring solution in this connection is continually increasing; for as our knowledge of the physiology and morphology of bacteria becomes more extended from day to day, new factors arise which have to be reckoned with in these investigations.

In a subsequent chapter we shall refer to the action of light on micro-organisms. Whilst we have already learnt how great is the effect of temperature upon the

vitality of bacteria, and have seen that the composition of the water into which they are introduced is of cardinal importance, we have so far omitted from the difficulties besetting the investigator perhaps the most troublesome of all, viz. the individual characteristics possessed by micro-organisms, and not only exhibited by different varieties, but even by the different individuals in one and the same cultivation, rendering it essential that in all experiments the previous history, as far as possible, of the organisms under observation should be recorded.

In the following tabulated series of researches on the behaviour of pathogenic bacteria in various waters we have endeavoured to give in all cases as fully as possible the essential details of the experiments as described by the authors themselves, together with the references to the original memoirs in which they are recorded. The following is a list of the micro-organisms subsequently dealt with :—

1. Bacillus typhosus-abdominalis (Typhoid Bacillus)
2. Spirillum cholerae asiaticae
3. Bacillus anthracis
4. Bacillus tuberculosis
5. Staphylococcus pyogenes-aureus
6. Streptococcus pyogenes
7. Streptococcus erysipelatis
8. Micrococcus tetragenus
9. Bacillus of mouse septicæmia
10. Bacillus of rabbit septicæmia
11. Bacillus of fowl cholera
12. Bacillus of swine plague
13. Bacillus of glanders
14. Bacillus of pneumonia
15. Bacillus of Finkler-Prior
16. Bacillus pyocyaneus
17. Aspergillus flavescens
18. Bacillus of tetanus

The mode of procedure usually adopted by the various investigators is to ascertain at different intervals of time, generally by means of plate-cultures, whether the organism is still present in the water in a vital condition. By this means information is also obtained as to the decrease or increase which has taken place in the numbers present during residence in the water.

This method, reasonable enough as it may appear, is not without certain objections, for in the first place the quantity of water which can be thus examined is relatively very small, and it is highly probable, therefore, that if only a few individual microbes were present in the water they might very easily escape detection altogether; whilst, secondly, the bacteria may, during their residence in the water, have their vitality so far enfeebled that they are unable to grow on a gelatine-plate, which often proves a somewhat adverse medium for the development of micro-organisms in a weakly condition.

Straus and Dubarry, and Percy Frankland in his later experiments on anthrax in water, have made a practice in particular cases of finally adding nutritive broth to the various samples of water. In this manner, if there are only a few microbes still remaining alive in the water, they will, on the addition of the broth, undergo abundant multiplication, and their presence can then be easily revealed by subsequent plate-cultures. Although in this manner we lose the means of estimating the actual numbers in which the particular pathogenic bacteria under observation are present in the water, we acquire more exact information as to the real duration of their vitality.

In addition to the question of vitality, it is of course also of the highest importance to ascertain whether the bacteria have retained their virulence or not, but this can only be done in the case of those bacteria which produce powerfully pathogenic effects on animals. Reference to the experiments which have been carried out in this direction will also be found embodied in the following tabular records of these investigations on the vitality of pathogenic bacteria in water.

Vitality of the Typhoid Bacillus in Water

The accompanying figures represent the appearance of the typhoid bacillus in microscopic and stained preparations. Fig. 19 shows the bacillus surrounded by



Fig. 18.—Typhoid Bacilli from a Pure Culture.
(After Jaksch.)

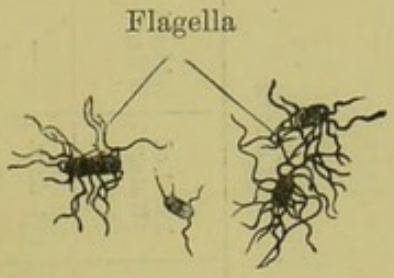


Fig. 19.—Typhoid Bacilli.
Magnified 1,100 times. (After Löffler.)

the flagella, or organs of locomotion. (See p. 54). On p. 410 in the appendix will be found a tabulated description of the more important morphological and other characters exhibited by the typhoid bacillus.

TABLE I.

Vitality of the Typhoid Bacillus in Various Waters

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Braem[1] (1889) .	—	—	—	—	—	188 days.	—	—	* Sterilised distilled water.
Freytag[2] (1890) .	—	—	—	—	—	—	—	5 months	Concentrated salt solution.
Giaxa[3] (1889) .	Two days old agar-agar culture grown at 36° C.; 1 needle-point taken.	—	—	—	—	—	—	Still present on the 9th day.†	† Unsterilised sea-water.
	Two drops broth-culture 8 days old and kept at 36° C.	—	—	—	—	—	—	Still present in large numbers on the 25th day.‡	‡ Sterilised sea-water.
Heraeus[4] (1886) .	Small quantity taken from a 'streaked culture.'	37° C.	Multiplied from 2 millions to 160 millions in 2 days.*						* Unfiltered River Spree water sterilised.
		12° C.	Multiplied from 12,000 to 87,000 in 2 days.*						

Hochstetter [5] (1887).	Potato-cultures grown for 4 and 7 days at temperature of room and at 36° C. Portions of the growth were mixed with sterilised distilled water and inoculated into the various waters.*	12°–15° C.	—	—	Longest duration of vitality observed, 7 days.†	Longest duration of vitality observed, 5 days.§	Longest duration of vitality observed, 5 days.¶	—	* Hochstetter states that he could detect no difference in the behaviour of the cultures grown at different temperatures. † Sterilised Berlin tap-water. § Sterilised distilled water. ¶ Seltzer-water.
Hueppe [6] (1887) .	—	10°–20° C.	—	—	20 to 30 days.*	—	—	—	* Sterilised Wiesbaden tap-water. † Unsterilised polluted well-water. † Ditto.
	Taken from potato-cultures 5 days old.	15°–20° C.	Over 30 days; none found, however, on the 60th day.†						
	—	10° C.	Rapid diminution in 2 out of 5 experiments, none were found on the 10th day.†						
Karlinski [7] (1889).	—	8° C.	—	6 days *	—	—	—	—	* Unsterilised Innsbruck drinking water
Kraus [8] (1887) .	—	10·5° C.	—	5 to 7 days, no longer demonstrable on 7th day.*	—	—	—	—	* Unsterilised well-water. † Unsterilised Munich Mangfall water. Considered a very pure water.
	—	10·5° C.	—	No longer demonstrable on 7th day.†					

[1] 'Untersuchungen über die Degenerationserscheinungen pathogener Bakterien im destillirten Wasser,' *Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, vol. vii. p. 11. [2] 'Ueber die Einwirkung concentrirter Kochsalzlösungen auf das Leben von Bakterien,' *Archiv für Hygiene*, vol. xi., 1890, p. 60.
[3] 'Ueber das Verhalten einiger pathogener Mikroorganismen im Meerwasser,' *Zeitschrift für Hygiene*, vol. vi., 1889, p. 162.
[4] 'Ueber das Verhalten der Bacterien im Brunnenwasser,' *Zeitschrift für Hygiene*, vol. i., 1886, p. 193.
[5] 'Ueber Mikroorganismen im künstlichen Selterwasser,' *Arbeiten aus dem kaiserlichen Gesundheitsamte*, vol. ii., 1887, p. 1.
[6] 'Die hygienische Beurtheilung des Trinkwassers vom biologischen Standpunkte,' *Schilling's Journal für Gasbeleuchtung und Wasserversorgung*, 1887. Separat-Abdrück, p. 130. [7] 'Ueber das Verhalten einiger pathogener Bakterien im Trinkwasser,' *Archiv für Hygiene*, vol. ix., 1889, p. 113.
[8] 'Ueber das Verhalten pathogener Bakterien im Trinkwasser,' *Archiv für Hygiene* vol. vi., 1887, p. 234.

TABLE I.—*continued*

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Maschek [1] (1887) .	—	18°–22° C.	—	—	10 to 80 days.*	—	—	—	Leitmeritz town water sterilised.
Mattei and Stagnitta [2] (1889)	—	8°–12° C.	—	—	4 days.				
Meade Bolton [3] (1886).	Small quantities taken from either sloped agar-agar or gelatine cultures and mixed with sterilised ordinary salt solution from which a few drops were taken and mixed with 10 c.c. of the water under investigation.	20° C.	Over 40 days.†	—	Over 7 days.§	From 2–3 and 10–14 days. None were found between 30 and 40 days.*	—	—	* Sterilised distilled water. † Highly polluted well-water sterilised. § Ordinary Göttingen water supply and containing very little organic matter. Sterilised.
		35° C.	From 10–14 days. None found after 20–24 or 30–40 days.†	—	—	From 2–3 days. None found after 6-7or10-14 or 20–24 days.*			
Pfeiffer [4] (1886) .	—	—	—	—	Upwards of 4 months.*	—	—	—	* Sterilised well-water.
Slater [5] (1893) .	Culture on agar, 37° C., 24 and also 48 hours old, inoculated into sterile distilled or sterile soda-water, 1·5 to 2 c.c. of which were employed for each inoculation.	Ordinary temperature.	—	—	—	Alive 50 days after inoculation.†	11 days, not found on the 13th day.* Dead on 5th day.‡ 8 days, dead on the 9th day.§	—	† Sterile distilled water. * Simple aërated, non-sterile. ‡ Sterile soda-water. § Sterile soda-water. non-aërated.

Straus and Dubarry[6] (1889)	One needle-point of a potato culture of the bacillus introduced into 10 c.c. of the water under examination.	20° C.	—	—	32 days.* 43 days.†	30–35 days.§	—	—	* Sterilised Ourcq water. † Sterilised Vanne water. The latter has less organic matter than the Ourcq water. § Sterilised distilled water.
		25° C.	—	—	81 days.*	69 days.§			
		35° C.	—	—	37 days.*	27 days.§			
Uffelmann[7] (1888)	—	Ordinary temperature of a room.	—	2 weeks.*	—	—	—	—	* Well-water in Rostock, unsterilised.
Wolffhügel and Riedel[8] (1886).	One needle-point from a gelatine culture introduced into 50 c.c. of the water.	18°–22° C.	—	—	Over 32 days.*	—	—	—	* Sterilised Berlin tap-water. † Sterilised distilled water. § Sterilised highly polluted river Panke water.
	One needle-loop from a broth-culture.	15°–20° C.	—	—	—	Over 15 days.†			
	One needle-point from a gelatine-culture introduced into 10 c.c. of the water.	35° C.	Over 10 days.§						

[1] 'Bakteriologische Untersuchungen der Leitmeritzer Trinkwässer,' *Jahresbericht der Oberrealschule zu Leitmeritz*, 1887.
[2] 'Sur la manière d'être des microbes pathogènes dans l'eau courante,' *Annali dell 'Istituto d'Igiene speriment. di Roma*, 1889.
[3] 'Ueber das Verhalten verschiedener Bakterienarten im Trinkwasser,' *Zeitschrift für Hygiene*, vol. i., 1886, p. 76.
[4] 'Die Beziehung der Bodencapillarität zum Transport von Bacterien,' *Zeitschrift für Hygiene*, vol. i., 1886, p. 398.
[5] 'Investigation of artificial mineral waters,' *Journal of Pathology and Bacteriology*, vol. i., 1893, p. 468.
[6] 'Recherches sur la durée de la vie des microbes pathogènes dans l'eau,' *Archives de Médecine expérimentale et d'Anatomie pathologique*, vol. i., 1889, p. 5.
[7] 'Trinkwasser und Infectionskrankheiten,' *Wiener medicinische Presse*, 1888, No. 37 (*Centralblatt für Bakteriologie*, vol. v., 1889, p. 89).
[8] 'Die Vermehrung der Bacterien im Wasser,' *Arbeiten aus dem kaiserlichen Gesundheitsamte*, vol. i., 1886, p. 455.

The results obtained by Kraus with unsterilised waters are given in greater detail in the following table, in which the typhoid bacilli are shown to have either disappeared or, at any rate, to have been no longer demonstrable when the ordinary water bacteria began to assert themselves. No special methods were, however, adopted for the separate identification of the typhoid organism in the presence of the ordinary water bacteria.

Typhoid Bacillus

Description of Water	Number of Days after Inoculation when Examined							
	1	2	3	5	7	9	20	150
	Number of Typhoid Bacilli found in 1 c.c. of Water							
(1) Munich water supply (Mangfall) . .	57,960	50,400	15,680	9,000	0	0	0	0
(2) Well-water, Munich .	57,000	50,840	32,643	8,900	0	0	0	0
(3) „ „ .	56,000	35,910	10,010	7,060	0	0	0	0
	Number of Water Bacteria found in 1 c.c. of Water							
(1) Munich water supply (Mangfall) . .	0	0	0	80	288,000	400,000	970,000	1,080
(2) Well-water, Munich .	0	0	490	Lost	300,000	427,000	innumerable	1,980
(3) „ „	0	0	280	500	256,000	Lost	456,000	1,050

From Table I. above it will be seen that a very considerable amount of attention has been given by numerous investigators to this all-important question of the vitality of typhoid bacilli in water. With regard to the behaviour of these micro-organisms in sterilised waters there is almost complete unanimity, and there can be no doubt that in these, even in sterilised distilled water, the bacilli will retain their vitality over considerable periods of time, at any rate for upwards of one month, although, of course, it is quite possible that in individual cases (depending on the strength or weakness of the cultures employed) they may perish in a shorter period of time.

Of far greater importance from a practical point of view is the deportment of the typhoid bacilli in un-

sterilised water, and on this head a great deal of the work done is unfortunately quite unreliable. It has already been pointed out (see p. 267) how essential it is to employ special methods for the detection of typhoid bacilli in natural waters, owing to the impossibility of recognising them with ease and certainty on an ordinary plate-culture containing numerous other colonies; it is, of course, equally important to employ these special methods in looking for typhoid bacilli in purposely infected unsterilised waters, and this has, unfortunately, only been done in very few of the researches the results of which are tabulated above.

There cannot be the slightest doubt that, if only the ordinary method of plate-cultivation is employed in such investigations on unsterilised water, the typhoid bacilli will be generally overlooked unless they are present in large numbers. Again, the attempts which have been made by some experimenters to count the typhoid colonies on such mixed plates, and the numerical estimates given of the typhoid bacilli in such unsterilised waters, must be wholly illusory, for the number of typhoid colonies which develop what may be called a typical appearance (*i.e.* one which enables them to be readily diagnosed with reasonable certainty) depends on a variety of different circumstances, amongst which may be mentioned the age of the plate, the extent to which the colonies are crowded together on the plate, very probably, also, the nature of the other colonies on the plate, and certainly the degree of vitality possessed by the typhoid bacilli themselves. Thus most of the investigators in question rely for their diagnosis of the typhoid colonies on mixed plates on the characteristic surface expansion-colonies of the typhoid bacilli, but nothing is commoner than to find only a vanishing proportion of the total number of

typhoid colonies on a plate giving rise to these surface-expansions at all, so that any estimate of the number of typhoid colonies from the number of such surface-expansion colonies would be utterly fallacious. In our own experiments, which are still in progress, on the vitality of typhoid bacilli in unsterilised waters, we have, therefore, invariably, in examining the waters for typhoid bacilli, submitted them to a preliminary process of sifting by Parietti's method (see p. 272) of phenol-broth culture, whereby the recognition of the typhoid bacillus, even when surrounded by a motley crowd of water-bacteria, is rendered possible. Of course this method of procedure banishes the possibility of forming any estimate of the actual number of typhoid bacilli in the water under investigation, although some idea of the numbers and degree of vitality of the bacilli in the water can be obtained from the length of time which elapses between the inoculation of the phenol-broth and the appearance of turbidity in it.

Vitality of the Cholera Bacillus in Various Waters

In the Appendix, p. 399, will be found a more detailed account of the principal microscopical and morphological characteristics of Koch's cholera bacillus; the following fig. 20 represents the bacilli as seen under the microscope.

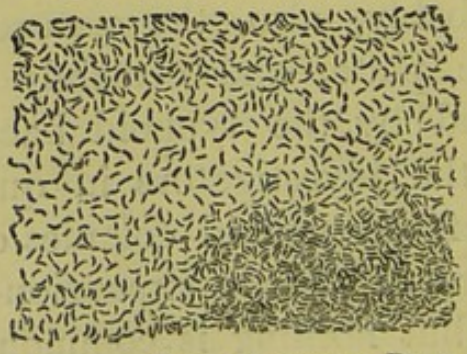


Fig. 20.—Cholera Bacilli from a Pure Culture.
(After Jaksch.)

TABLE II.

Vitality of the Cholera Bacillus in Various Waters (Spirillum choleræ asiaticæ) (Koch)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Waters	Sea Water or Concentrated Salt Solution	Remarks
Babes [1] (1885) .	—	—	—	7 days.*	—	1 day.	—	—	* Berlin tap-water.
Braem [2] (1889) .	—	—	—	—	—	24 hours.*	—	—	* Sterilised distilled water.
Frankland, Percy [3] (1886).	One or more needlefuls of a gelatine-culture (in which the bacillus only grew feebly) were introduced into about 50 c.c. of sterilised distilled water, from which some drops were inoculated into the waters under examination.	20° C.	None discoverable on the 2nd day.†	—	None discoverable on the 2nd day.	None discoverable on the 2nd day.*†	—	—	* Sterilised distilled water. † The cholera bacillus was in a weakened condition, as shown by its feeble growth on gelatine. ‡ Sterilised London sewage. § Sterilised deep-well water obtained from the chalk. §§ Sterilised filtered Thames water. §§§ Extensive multiplication of the comma bacilli took place during this period.
		35° C.	Either at 20° or 35° C.‡	—	Either at 20° C. or 35° C.†§	Either at 20° or 35° C.*†			
	Instead of a gelatine-culture the bacillus was taken from a vigorous growth in broth and similarly diluted with distilled water, before inoculation into the waters under examination.	20° C.	Over 11 months at 20° C.‡§§§	—	Present on the 9th day, but none found on the 17th day. The numbers were more reduced in the samples kept at 35° C. than at 20° C.§				
		35° C.		—	None were discoverable at 35° C. on the 5th day. Present in small numbers on the 9th day at 20° C. §§				

[1] 'Untersuchungen über R. Koch's Kommabacillus.' Virchow's *Archiv*, 1885, p. 152.

[2] Foot-note (No. 1) to Table I., p. 291.

[3] 'On the Multiplication of Micro-organisms.' *Proc. Roy. Society*, 1886, p. 526.

TABLE II.—*continued*

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Freytag [1] (1890) .	—	—	—	—	—	—	—	6–8 hours.*	* Concentrated salt solution.
Gärtner [2] (1888) .	—	11·5° C.	—	None found on the 2nd day.*	—	—	—	—	* Unsterilised spring water.
Giaxa [3] (1889) .	One drop of a 4-day-old bouillon culture kept at 37° C.	—	—	—	—	—	—	Not discoverable on the 4th day.* More than 36 days.†	* Unsterilised sea-water. † Sterilised sea-water.
Hochstetter [4] (1887).	Broth-cultures. 0·1 c.c. bouillon : 50 c.c. water.	About 18° C.	—	—	267, 382, 392 days.‡	24 hours to 7 days.†	3 hours.*	—	* Unsterilised seltzer-water. † Sterilised distilled water. ‡ Sterilised Berlin tap-water.
Hueppe [5] (1887) .	—	10° C. 16°–20° C.	Once out of 8 experiments found in small numbers on 20th day.† Up to 60 days.†	— —	Over 10 days.* 30 days.*	—	—	—	* Sterilised Wiesbaden tap water. † Sterilised polluted well-water.
Karlinski [6] (1889)	—	8° C.	—	3 days.*	—	—	—	—	* Unsterilised Innsbruck drinking water
Koch [7] (1887) .	—	—	6–7 days.†	Over 30 days.*	—	—	—	—	* Well water. † Berlin (Canal) water. It is not stated if these were sterile or not.
Kraus [8] (1887) .	—	10·5° C.	—	None found on the 2nd day.*	—	—	—	—	* Munich Mangfall water supply and water from 2 pump wells in Munich

					days.†				added to the distilled water, but the exact quantity is not stated. † Sterilised Leitmeritz town water.
Nicati & Rietsch[10] (1885).	—	—	32 days.§	—	—	More than 20 days.*	—	81 days.† 64 days.‡	* Sterilised distilled water; a comparatively large amount of culture-fluid was introduced. † Old harbour water at Marseilles, sterilised. ‡ Sea-water, sterilised. § Bilge water from the bottom of a ship, sterilised.
Pfeiffer [11] (1886).	—	—	—	—	More than 7 months.*	—	—	—	* Well-water, sterile.
Ringeling [12] (1886)	—	—	32–37 days.*	—	—	—	—	—	* Bilge water which had accumulated in the hold of a ship, doubtless sterilised.
Slater [13] (1893) .	Culture on agar, 37° C., 48 hours old, inoculated into sterile distilled or sterile soda water. 1·5–2 c.c. were employed for each inoculation.	Ordinary temperature	—	—	—	5 hours, dead at the end of 1 day.*	½–1 hour.† 1–1½ hour.‡ 9 hours, dead at the end of 1 day.§	—	* Sterilised distilled water. † Simple aërated non-sterile water. ‡ Sterile soda-water. § Non-aërated sterile soda-water. In another experiment it survived 9 days, but was extinct on the 10th day. The organism was obtained from a different source in the latter case.

[1] Foot-note (No. 2) to Table I., p. 291. [2] *Untersuchung des Wassers*, by Tiemann and Gärtner, 1889, p. 595. [3] Foot-note (No. 3) to Table I., p. 291.
[4] *Loc. cit.*, foot-note (No. 5) to Table I., p. 291. [5] Foot-note (No. 6) to Table I., p. 291. [6] Foot-note (No. 7) to Table I., p. 291.
[7] 'Conferenz zur Erörterung der Cholerafrage.' *Berliner klinische Wochenschrift*, 1887, No. 37*a* and *b*. (Tiemann and Gärtner, *Loc. cit.*, p. 593.)
[8] Foot-note (No. 8) to Table I., p. 291. [9] Foot-note (No. 1) to Table I., p. 293.
[10] 'Expériences sur la vitalité du bacille virgule cholérigène.' *Revue d'Hygiène*, 1885, No. 5. [11] Foot-note (No. 4) to Table I., p. 293.
[12] 'Bijdrag tot de kennis van het ruimwater van schepen.' *Dissertation*, Amsterdam, 1886. ['Die hygienische Beurtheilung d. Trinkwassers.' Hueppe, *Schilling's Journal*, 1887].
[13] Foot-note (No. 5) to Table I., p. 293.

TABLE II.—*continued*

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry [1] (1889) .	One needle-point of a cholera culture introduced into 5 c.c. of the water under examination.	20° C.	—	—	26 days.* 39 days.†	14 days.‡	—	—	* Sterilised Ourcq canal water. † Sterilised Vanne spring water, a purer water than the above. ‡ Sterilised distilled water.
		35° C.	—	—	30 days.*	Perished within 24 hours.‡			
Wolffhügel and Riedel [2] (1886) .	A needle-point or needle-loop of broth cultures into 50 c.c. of the water under examination.	16°–22° C.	More than 7 months.* More than 7 months.† 20 days.§§	More than 20 days.‖	More than 7 months.§ More than 7 months.‡	None were demonstrable on the 1st day after the flasks were inoculated.‖‖ On one occasion, however, they were found after 33 days, which the authors themselves state as being an exceptional occurrence, probably due to some impurity in the distilled water.	—	—	* Sterilised River Panke water in Berlin. † Sterilised River Spree water. ‡ Sterilised well-water. § Sterilised Berlin tap-water. §§ Unsterilised River Spree water. ‖ Unsterilised Berlin tap-water and well-water. ‖‖ Distilled water, but whether sterile or not is not stated.

[1] Foot-note (No. 6) to Table I., p. 293.

[2] Foot-note (No. 8) to Table I., p. 293.

In the following table are recorded more in detail the results obtained by Kraus on introducing cholera bacilli into three different unsterilised waters at Munich :—

Cholera Bacillus

Description of Water	Number of Days after Inoculation when Examined				
	1	2	4	8	135
	Number of Cholera Bacilli found in 1 c.c. of Water				
(1) Munich water supply (Mangfall) . .	10,100	0	0	0	0
(2) Well-water, Munich .	8,700	0	0	0	0
(3) ,, ,,	9,420	0	0	0	0
	Number of Water Bacteria found in 1 c.c. of Water				
(1) Munich water supply (Mangfall)	30	400	70,000	1,400,000	2,040
(2) Well-water, Munich .	80	900	85,000	innumerable	8,100
(3) ,, ,,	250	2,000	100,000	innumerable	4,100

In connection with the vitality of the cholera bacillus in water, some recent investigations made by Trenkmann[1] are of considerable interest. In these researches varying doses of different salts were added to a particular well-water kept at from 21°–24° C. ; the cholera bacillus was introduced, and its subsequent behaviour carefully watched by means of plate-cultures. In some experiments the water was sterilised, whilst in others it was used in its natural condition, and the competition between the ordinary water bacteria with the cholera bacilli was noted.

One, two, or three drops, as the case might be, of a 10-per-cent. solution of sodium chloride, sodium nitrite, sodium nitrate, sodium carbonate, disodium phosphate, also potassium chloride, potassium nitrate, sodium sulphide, were added to 10 c.c. of the water contained in test-tubes.

These salts were selected as being those which might normally be present in waters of different kinds, although even the lowest proportions in which they

[1] 'Beitrag zur Biologie des Kommabacillus,' *Centralblatt für Bakteriologie*, vol. xiii., 1893, p. 313.

were added by Trenkmann exceeds that generally present in ordinary drinking waters.

Again, in the inoculation of the cholera organism considerable quantities of organic material must also have been introduced into the waters, for the bacilli were abstracted *direct* from broth-cultures by means of a needle-loop, in which manner, of course, an appreciable amount of broth was also introduced, and the character of the water materially altered. This error is one, as we have already pointed out, which has been unfortunately made by many investigators, and has greatly detracted from the value of the results obtained.

The following table may be cited as an example of the mode of investigation pursued, and of the results obtained :—

TABLE I.—*Sterile Well water*

The Waters were Maintained at 21–24° C.	Number of Cholera Bacilli in 1 Needle-loop †	
	After 24 hours	After 8 days
(1) 10 c.c. sterile well-water	580 520	5
(2) ,, +1 drop * 10 per cent. sodium chloride	6,120	12,480
(3) ,, +2 drops ,, ,, ,,	9,240	19,560
(4) ,, +3 ,, ,, ,, ,,	15,000	10,440
(5) ,, +1 drop ,, sodium nitrite .	1,740	10,920
(6) ,, +2 drops ,, ,, ,,	6,600	1,460
(7) ,, +3 ,, ,, ,, ,,	17,160	2,260
(8) ,, +1 drop ,, sodium nitrate .	8,040	4,040
(9) ,, +2 drops ,, ,, ,,	6,660	14,760
(10) ,, +3 ,, ,, ,, ,,	20,940	16,080
(11) ,, +1 drop ,, disodium phosphate	3,360	—
(12) ,, +2 drops ,, ,, ,,	7,560	—
(13) ,, +3 ,, ,, ,, ,,	6,540	—
(14) ,, +1 drop ,, sodium carbonate .	7,440	—
(15) ,, +2 drops ,, ,, ,,	28,680	—
(16) ,, +3 ,, ,, ,, ,,	31,560	—
(17) ,, +2 drops 10 per cent. sodium chloride + 1 drop 10 p.c. disodium phosphate	54,720	—

On the control-plate, poured immediately after the introduction of the bacilli, there were {1,440 / 1,880} colonies of cholera bacilli.

* 25–27 drops = 1 c.c.

† It is much to be regretted that this investigator has departed from the time-honoured custom of determining the number of bacteria in 1 cubic centimetre, and has substituted for this the irrational and ephemeral unit of volume represented by the drop of liquid contained in a particular needle-loop in his possession.

From these experiments it appears that in those waters to which no additions of salt had been made the cholera bacilli rapidly diminished in numbers, whilst in the others their multiplication took place almost in proportion to the quantity of salt added, this being especially marked in the case of No. 17.

It was also found that the addition of potassium salts stimulated the vitality of this bacillus in a marked degree, three drops of a 10-per-cent. solution of potassium chloride inducing an increase in the numbers present after twenty-four hours from 1,020 to 43,320 in the needle-loop.

Experiments were also made with unsterile water, as will be seen from the following table :—

TABLE II.—*Unsterile Well-water*

The Waters were Maintained at 21–24° C.	Number of Organisms in 1 Needle-loop after 24 hours	
	Cholera Bacilli	Water Bacteria
(1) 10 c.c. unsterile well-water	216	19,300
(2) ,, +1 drop 10 per cent. sodium sulphide .	0	2,370
(3) ,, +1 ,, ,, sodium chloride .	54	30,000
(4) ,, +2 drops ,, ,, ,,	1,940	32,400
(5) ,, +3 ,, ,, ,, ,,	10,000	32,400
(6) ,, +3 drops 10 per cent. sodium chloride + 1 drop 10 per cent. sodium sulphide	5,500	3,240
(7) ,, +3 drops 10 per cent. sodium chloride + 2 drops 10 per cent. sodium sulphide	17,100	540
(8) ,, +3 drops 10 per cent. sodium chloride + 3 drops 10 per cent. sodium sulphide	28,100	2,160

The author has unfortunately omitted to state the number of cholera bacilli introduced into this water, but from his remarks it would appear that the initial number must have been largely in excess of 216 per needle-loop.

Thus in the unsterile water the cholera bacilli quickly diminish, whilst the water bacteria rapidly multiply. The addition of sodium chloride markedly stimulates the vitality of the water bacteria, so much so that when only one drop was added the cholera

bacilli were nearly overwhelmed by them, the latter, however, with larger additions of the salt were rendered more capable of holding their own in competition with the water microbes. Sodium sulphide acts prejudicially, as might be anticipated, on both the water forms and the cholera bacilli, but the vitality of the latter is most distinctly increased by the addition of sodium chloride to the sodium sulphide; on the other hand the water bacteria are not favourably influenced by this addition.

Experiments were also made on the effect of adding sodium chloride in conjunction with sodium carbonate to this unsterile well-water, and it was found that whilst a large and rapid increase took place in the water bacteria, the cholera organisms, after holding out a few days, disappeared entirely on the fourth day; on the other hand, when sodium chloride, sodium carbonate, and sodium sulphide were added together, the cholera bacilli multiplied extensively side by side with the water microbes, and even after seven days the former were demonstrable, although only two colonies were found on the plate.

Investigations at a lower temperature ($12\frac{1}{2}°$–16° C.) were also made with unsterile water with various additions of salts, and it was found that in only two instances could the cholera bacilli be detected after nine days, and then only in those waters to which sodium chloride, disodium phosphate, and sodium sulphide had been added together.

Trenkmann states that in all his investigations with this unsterile water he found that an addition of sodium chloride and sodium sulphide caused a rapid disappearance of the majority of the different kinds of water bacteria present, sometimes only one variety remaining in competition with the cholera organism; such sur-

viving varieties, however, underwent extensive multiplication.

These results are of much interest, if we remember that the cholera organism has been found capable of living for long periods in brackish water, such as the harbour water at Marseilles. Still more recently this has been proved in the case of the Hamburg water supply, which was found on analysis,[1] during the cholera epidemic of 1892, to be distinctly brackish in character. The following table shows the composition of this water as revealed by chemical analysis :—

Sample of Hamburg Water received from Mr. Ernest Hart, October 21, 1892

Results of Analysis expressed in parts per 100,000

Total Solid Matters	Organic Carbon	Organic Nitrogen	Ammonia		Nitrogen as Nitrates and Nitrites	Chlorine	Hardness		
			Free	Albuminoid			Temporary	Permanent	Total
78·00	·926	·088	·030	·047	0	31·3	4·1	13·7	17·8

Oxygen consumed by organic matter, as measured by reduction of a solution of permanganate acting for three hours in the cold = ·366.

The water was very turbid, depositing a quantity of brown suspended matter.

This high percentage of salt is due to the waste liquors which are discharged from the Stassfurt salt works and other factories into the Elbe and its tributaries. In Magdeburg, where filtered Elbe water is used, the percentage of salt was so high during December 1892 that the water was not only unpalatable but unusable.[2] Hueppe,[3] who made a special study of the

[1] Percy Frankland, *British Medical Journal*, 1893, p. 251.

[2] 'Ueber den Einfluss stark salzhaltigen Elbwassers auf die Entwickelung von Cholerabacillen,' Aufrecht, *Centralblatt für Bakteriologie*, vol. xiii., 1893, p. 353.

[3] *Die Choleraepidemie in Hamburg*, 1892, p. 20. Berlin, 1893.

cholera epidemic in Hamburg, points out that during the latter half of August 1892 the river at Hamburg sank to the lowest ebb which had been known for many years, consequently the organic matter, together with the salts present, were in an unusually concentrated condition.

Knowing as we now do that the cholera organism is placed at the greatest advantage when a high percentage of salt is present, the particularly brackish condition of the Elbe during the cholera epidemic of 1892 is of special interest and importance.

From what has been said above, it will be seen that the cholera spirillum is far more susceptible to immersion in water than is the typhoid bacillus. Thus the experiments conclusively show that in distilled water the cholera spirilla are rapidly destroyed, generally within twenty-four hours, the few exceptional cases in which they have been observed (Nicati and Rietsch, Wolffhügel and Riedel) to survive for longer periods of time being almost certainly due to appreciable quantities of food-materials having been imported into the distilled water along with the spirilla themselves. There appears to be but little doubt that this speedy destruction of these bacteria in distilled water is due to the rapid osmosis which must take place, for it has been shown (Maschek) that if a certain proportion of common salt be added to the distilled water they may survive for a long period of time; thus a survival of forty days is recorded under these circumstances. Similarly, even in those potable waters which, like that from deep wells in the chalk, contain only a mere trace of organic matter (see Percy Frankland, above), their longevity was much greater than in distilled water, thus substantiating the view that the rapid destruction in distilled water is due rather to the low

specific gravity of the latter than to the absence of organic food-materials.

In ordinary potable waters, when sterile, the cholera spirilla retain their vitality for periods of time varying from a few days to several months.

In these sterile potable waters, the cholera bacilli do not appear to undergo any extensive multiplication, but a curious phenomenon has been observed in this connection both by Wolffhügel and Riedel and by ourselves, for on infecting such waters with cholera spirilla we found that their numbers, as revealed by plate-cultivation, underwent rapid reduction during the first few days, which decline was subsequently followed by a slight but appreciable increase, soon followed again by a subsequent diminution. The probable explanation of this would appear to be that a number of the spirilla soon perish on being introduced into the water, and that those hardier forms which survive then undergo slight multiplication, possibly at the expense of the food-materials set at liberty by their fallen comrades; whilst it may also be due to the spirilla adapting themselves, to a certain extent, to the aqueous medium, for Wolffhügel and Riedel found that on transferring these cholera spirilla which had undergone this slight multiplication to a fresh quantity of water, they continued to multiply appreciably in this also; the results were, however, not so uniform as to admit of this hypothesis being accepted without further investigation which the importance of the subject certainly demands.

On the other hand, in sterile sewage, the extensive multiplication of the cholera bacilli admits of no doubt whatever, for in this material we found the organism present in large numbers, even after eleven months, enormous multiplication having taken place in the interval.

As regards the behaviour of the cholera spirilla in unsterilised waters, the criticism which we offered above in connection with the same problem concerning the typhoid bacilli, applies with equal force here, for unless special methods (see p. 276) be adopted for the discovery of the cholera bacilli in unsterilised waters, there can be no doubt that they will in many cases be entirely overlooked. Unfortunately, no such special methods of detection have been employed in the case of any of the experiments recorded above on the cholera bacilli in unsterilised water. The results of these experiments, which must therefore be accepted with considerable reserve, indicate that in unsterilised water the vitality of the cholera spirilla is of the very briefest duration (according to Kraus they had disappeared on the second day), which is obviously difficult to reconcile, or even quite out of harmony, with the indisputable facts concerning the communication of cholera by drinking water. It is also difficult to believe in such rapid destruction of the cholera bacilli taking place after their presence has now been on a number of occasions demonstrated by the special methods in question in various polluted natural waters, and we cannot help thinking that if these special methods had been employed in such experiments as those of Kraus, the cholera bacilli would have been discovered after a much longer period of time. In this connection we have always regarded as of great importance some observations made by Gruber, in which such special methods were used ('Wiener medicinische Wochenschrift,' 1887, Nos. 7 and 8). In these experiments he showed that when the cholera spirilla are mixed with putrefactive bacteria, 'although the latter gain an enormous numerical ascendency over the comma spirilla

for some time, yet the vitality of the latter is by no means extinguished, for if the struggle between the two be sufficiently protracted, until the process of putrefaction is less active, the presence of the comma bacilli can be again readily demonstrated by cultivation. It is necessary, therefore, to exercise considerable caution in judging upon this point in the present state of our knowledge, and it would be highly premature to place too much reliance upon this alleged destruction of pathogenic forms by non-pathogenic ones.'[1]

The recent experiments of Trenkmann, moreover, indicate that the preservation of the cholera bacilli in unsterile water may be materially influenced by the presence of salts, a circumstance which not improbably may have played an important part in the distribution of cholera by means of the waters of the Elbe and Thames, which during some of the London cholera epidemics was supplied to the metropolis from its tidal portion; whilst in the case of shallow well-waters which have given rise to outbreaks of cholera, large proportions of salts (nitrates, chlorides, and sulphates) will doubtless not have been wanting.

[1] 'Recent Bacteriological Research in connection with Water Supply.' Percy Frankland, *Journ. Soc. of Chem. Industry*, 1887.

Vitality of the Anthrax Bacillus and its Spores in Various Waters

The following fig. 21 represents the appearance of anthrax bacilli when seen in a microscopic preparation.

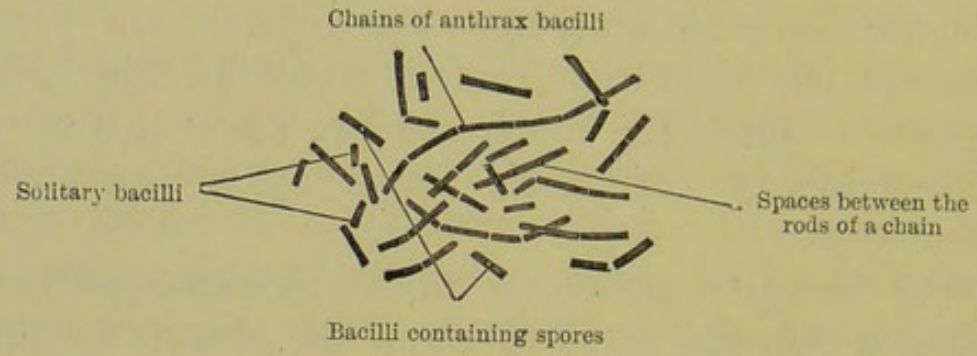


Fig. 21.—Bacilli of Anthrax.

For greater details concerning the various characteristics of this organism, see p. 416, in the Appendix.

Kraus has carried out similar experiments with anthrax to those described with typhoid and cholera bacilli, in which the effect on the pathogenic bacteria of competition with the native water forms was shown so prominently.

Thus :—

Sporeless Anthrax

Description of Water	Number of Days after Inoculation when Examined				
	1	2	4	8	130
	Number of Anthrax Bacilli found in 1 c.c. of Water				
(1) Munich water supply (Mangfall) . .	1,150	900	0	0	0
(2) Well-water, Munich .	1,050	1,000	0	0	0
(3) „ „	1,180	850	0	0	0
	Number of Water Bacteria found in 1 c.c. of Water				
(1) Munich water-supply (Mangfall) . .	50	800	10,400	1,500,000	2,100
(2) Well-water, Munich .	200	1,000	9,700	innumerable	7,250
(3) „ „	800	2,500	150,000	innumerable	4,240

These results have been since confirmed by Karlinski (see table III., p. 316), who, using unsterile water and sporeless anthrax, found that the latter had disappeared already on the third day.

In considering the vitality of anthrax in water, it is obvious that a sharp distinction must be made between the vitality of the bacilli and of the spores respectively. As regards the vitality of the spores, there is practically complete unanimity amongst the numerous investigators who have given attention to this matter. Thus in sterilised water, even distilled water, the spores of anthrax are almost indestructible, retaining both their vitality and virulence over many months, and probably even years. Excepting in very foul waters, such as sewage, however, no multiplication appears to take place; those instances in which multiplication has been recorded in potable waters are probably due to culture–material having been introduced into the water at the time of infection. In unsterilised waters, the anthrax spores, although exhibiting an endurance which may extend over several months, unquestionably undergo degeneration more rapidly than in sterile water; moreover, the nature of the water appears materially to affect this result, for in the experiments of one of us it was found that the anthrax spores underwent markedly more rapid destruction in unsterilised Loch Katrine (peaty moorland water) than in unsterilised Thames water. Again, in this matter temperature exercises an important influence; in fact, the degeneration in the Loch Katrine water was only observed at a summer (18°–20° C.) and not at a winter (6°–8° C.) temperature.

As regards the behaviour of anthrax *bacilli* in water there is more divergence amongst the results arrived at by different observers. In the first place, it must be

carefully borne in mind that the sporeless anthrax bacilli employed by different investigators have had totally different origins; thus, whilst some have used anthrax bacilli taken directly from the blood or organs of animals dead of anthrax, others have relied upon young artificial cultures of anthrax *believed* to be free from spores. It is possible that these totally different sources of the bacilli may account for some of the discrepancies which have arisen. There appears, however, to be no doubt that the anthrax bacilli themselves enjoy only a very short life when introduced into water, either sterile or otherwise; thus numerous observers have recorded their disappearance in the course of a few days. On the other hand, in some cases, although introduced into the water in the bacillar form only, anthrax has been still discoverable in the living state after months. As far as such cases have been further examined, however, it has been proved that the anthrax bacilli introduced into the water have formed spores, with the production of which the almost indefinite persistence of vitality is ensured. Moreover, that in some cases in which anthrax bacilli are introduced into water spores should be formed and in others not, appears to be mainly dependent on the temperature at which the water is preserved. Some recent experiments, conducted by one of us in conjunction with Dr. Templeman, of Dundee, place this matter in a very clear light.

In these experiments the spleen of a mouse dead of anthrax was broken up in a little sterile water, and a small quantity of this was then distributed in a large volume of steam-sterilised Thames water; the latter, after being thus infected, was divided up into a number of sterile test-tubes, some of which were placed in a dark cupboard (temperature 13° C.), others in a refrigerator (5° C.), and others in an incubator (19° C.).

On systematically examining these several tubes, it was found that on the fifth day, whereas anthrax was no longer discoverable in the tubes kept at 5° C., it was present in large numbers in those kept at 19° C., whilst in those which had been preserved at 13° C. it was still present, although in diminished numbers. Again, after fourteen days, all the tubes kept both at 5° C. and at 13° C. were found quite free from anthrax, whilst the tubes maintained at 19° C. yielded large numbers of anthrax colonies. That the anthrax in these latter tubes was present in the spore form was proved by the fact that they resisted heating to 70° C. for ten minutes.

These results clearly show that the anthrax bacilli underwent destruction in the course of a few days in the sterile Thames water which was kept at temperatures below that at which spore-formation can take place; whilst on the other hand the bacilli from the same source, in the same water, maintained at 19° C., gave rise to an abundant crop of spores, the vitality of which was indefinitely preserved. The precise temperature at which anthrax bacilli form spores possibly varies to some extent according to their origin and previous history, for in Meade Bolton's experiments, recorded in the table above, it appears that no spores can have been formed, although the water is said to have been preserved at 20° C.

What may be the behaviour in water of that variety of anthrax known as *asporogène*, and which is incapable of forming spores under any known conditions, has not yet been determined.

TABLE III.

Vitality of the Anthrax Bacillus in Various Waters (Bacillus anthracis)

Investigators and Date of Experiments	Source of Organism introduced into Water	Temperature at which Infected Water was maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Water	Sea-water or Concentrated Salt Solution	Remarks
Braem[1] (1889) .	—	—	—	—	—	12 days.*	—	—	* Sterilised distilled water.
Frankland, Percy[2] (1887)	A small quantity of an agar-agar culture, in which spores were present, was introduced into 50 c.c. of sterilised distilled water, from which 2 c.c. were introduced into 1,500 c.c. of the waters under examination.	15°–20° C.	Over 60 days * (great increase in numbers).	—	Over 60 days (no diminution in numbers).†	Over 60 days (no diminution in numbers).‡	—	—	* Sterilised London sewage. . † Sterilised London tap-water (River Thames). ‡ Sterilised distilled water.
Frankland, Percy,[3] Second series of investigations (1893)	Five loops of a 3 weeks' old agar-agar culture, in which spores were present, mixed with about 50 c.c. of sterilised Thames water. 2 c.c. of this introduced into about 2 litres of the water under examination. In another similar series of experiments a larger number of anthrax bacteria were introduced; in all cases the results were the same, except that in the latter the waters were more rapidly fatal to mice.	6°–10° C. and 18°–20° C. (No difference could be detected at the higher and lower temperature respectively).	—	Upwards of 7 months (great diminution in numbers).* Upwards of 7 months (great diminution in numbers).†	Upwards of 7 months (no diminution in numbers).‡	—	—	—	* Unsterilised and unfiltered River Thames water. White mice inoculated with 1 c.c. of this water, after the latter had been kept for 7 months, remained alive. Anthrax was present, however, in small numbers, for by revivifying them, by adding bouillon to the water, mice died of anthrax. † Unsterilised River Thames water, paper filtered. This water, without adding bouillon, after 7 months was inoculated into mice; the latter died of anthrax. ‡ The same water (Thames), whether sterilised by steam or by filtration through porous porcelain, after being kept for 7 months, was fatal to mice.

Ditto (1893).	Sporiferous anthrax taken from agar-agar culture 4 days old. Eight needle-loops mixed with 50 c.c. sterile water. 1 c.c. of this added to 750 c.c. of the water under examination.	6°–10° C.		at 18°–20° C.* Upwards of 3 months at 6°–10° C. (no diminution in numbers).†					unsterilised. Not fatal to mice on inoculation after 3 months. † Fatal to mice on inoculation after 3 months.
—	—	—	—	—	—	Upwards of 3 months ‡ (no diminution in numbers). Upwards of 3 months § (no diminution in numbers).	—	—	‡ Porcelain - filtered Loch Katrine water. Fatal to mice after 3 months. § Steam-sterilised Loch Katrine water. Fatal to mice after 3 months.
Ditto, experiments showing action of light on anthrax in water (1893)	—	—	—	Alive after 56 hours' insolation, but extinct after 84 hours' ditto.* Almost extinct after 92 hours' insolation; quite extinct after 151 hours' exposure.†	Alive after 56 hours' insolation, but extinct after about 84 hours' insolation.‡ Alive after about 56 hours' insolation; extinct after about 84 hours' insolation.§ Alive after exposure for rather more than 6 months to *diffused* daylight in all the Thames waters, whether sterilised or unsterilised.§§	—	—	—	* Unfiltered - Thames water. † Paper-filtered Thames water. ‡ Porcelain - filtered Thames water. § Steam - sterilised Thames water. In all cases inoculations of the various waters at the end of the experiment were made into white mice, but in spite of adding bouillon to the water, the mice all remained alive. §§ The unfiltered and paper-filtered waters were unable to kill mice directly, but only after the addition of broth did they become fatal. In the case of the filtered and steam-sterilised waters, the inoculation was directly fatal.
Gärtner [4] (1888).	Anthrax *bacilli*	12° C.	—	Up to 4 days. None found on the 6th day.*	—	—	—	—	* Unsterilised tap-water.

[1] See foot-note (No. 1) Table I., p. 291.
[2] 'Recent Bacteriological Research in connection with Water Supply.' *Society of Chemical Industry*, vol. vi. 1887.
[3] 'On the Vitality of Sporiferous Anthrax in Potable Waters.' *Proc. Roy. Society*, 1893, p. 177.
[4] 'Untersuchung des Wassers.' Tiemann-Gärtner, 1889, p. 588.

TABLE III.—*continued*

Investigators and Date of Experiments	Source of Organism Introduced into Water	Temperature at which Infected Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Water	Sea-water or Concentrated Salt Solution	Remarks
Hochstetter[1] (1887)	Anthrax *bacilli* taken from the blood of guineapigs dead of anthrax.	11°–20° C.	—	—	Upwards of 3 days; extinct on the 7th day.‡	Upwards of 3 days; extinct on the 7th day.†	15 mins. to 1 hour.*	—	* Unsterilised seltzer water. † Sterilised distilled water. ‡ Sterilised tap-water.
	Anthrax *spores*. Potato culture obtained from the blood of a guineapig which had died of virulent anthrax. The potato was kept for 5 days in the incubator at 30°C. to ensure the perfect development of the spores.	—	—	—	Upwards of 154 days.‡	Upwards of 154 days.†	Upwards of 154 days.*		These spores after 154 days' residence in these various waters were *equally* fatal to guineapigs as those immersed only for 24 hours.
					—	—			
Hueppe[2] (1887) .	Anthrax *bacilli* .	16° C.	—	—	None found on the 5th day.*	—	—	—	* Sterilised Wiesbaden tap-water.
Karlinski[3] (1889)	Anthrax *bacilli*. Blood was inoculated into the water from an animal dead of anthrax.	8° C.	—	None found on the 3rd day.*	—	—	—	—	* Unsterilised Innsbruck water-supply.
Koch[4] . . .	Anthrax spores .	—	—	—	—	One year.	—	—	—
Kraus[5] (1887) .	Anthrax *bacilli* .	10·5° C.	—	Up to 2 days. None found on the 4th day.*	—	—	—	—	* Unsterilised Munich water.
Meade Bolton[6] (1886)	Anthrax *bacilli*. *See* Table I. for the mode of experiment.	20° C.	—	—	Rapid diminution when examined after 55 hours. On the 6th day none were found.*	—	—	—	* Sterilised Berlin tap-water. † Distilled water,
—	—	35° C.	—	—	None were found at the end of 55 hours.*				
—	Anthrax *spores* .	20° C.	—	—	Innumerable after 90 days.*	Innumerable after 90 days.†			
—	—	35° C.	—	—	Upwards of 30 days. None found after 90 days.*	Upwards of 30 days. None found after 90			

Straus & Dubarry[3] (1889)	Anthrax *bacilli*. Blood taken on the point of a platinum needle from a guineapig dead of anthrax.	20° C.	—	—	28 days.* 65 days.†	24 to upwards of 131 days.‡	—	—	* Sterilised Ourcq water. † Sterilised Vanne water. ‡ Sterilised distilled water. These investigators inoculated anthrax *bacilli* into distilled water and kept it for 64 days at 20° C. This water was then heated for 10 minutes up to 65° to 70°, to destroy everything but possible spores. Bouillon was then added, and the sample again placed in the incubator at 30° C., when anthrax cultures were obtained, proving that the bacilli can produce spores in distilled water.
Uffelmann[9] (1888)	Anthrax *spores* .	Ordinary temperature of a room.	—	Upwards of 3 months.*	—	—	—	—	* Well-water in Rostock, unsterilised.
Ward[10] (1893) .	Ten c.c. of raw unsterile Thames water, in which a vigorous potato culture had been shaken up, were inoculated into 600 c.c. of raw unsterilised River Thames water. A good deal of starch was carried over with the inoculation.	12°–15° C In bright daylight, but no direct sunshine.	—	Upwards of 7 months.*	—	—	—	—	* Unsterilised River Thames water. Animals inoculated with 2 c.c. of this water died of anthrax in 5 days. † Boiled Thames water. One litre was taken and inoculated with 2 c.c. of the potato-infected water, so that water microbes were introduced with the anthrax. ‡ The water during this time became quite green with algæ. Guineapigs inoculated intra-peritoneally with 2½ c.c. of this water died of anthrax.
		At 20° C. for first 2 months, and at ordinary temperatures for 5 months. Kept in the dark.	—	Upwards of 7 months.†					
	300 c.c. of raw unsterile Thames water, inoculated with a large quantity of virulent anthrax from a potato culture. A great deal of starch was carried over.	Ordinary temperatures, in diffused light.	—	Upwards of 7 months.‡					

[1] See foot-note (No. 5) Table I., p. 291. [2] See foot-note (No. 6) Table I., p. 291. (Separat-Abdruck, p. 129.) [3] See foot-note (No. 7) Table I., p. 291.
[4] 'Untersuchung des Wassers.' Tiemann-Gärtner, 1889, p. 585. [5] See foot-note (No. 8) Table I., p. 291. [6] See foot-note (No. 3) Table I., p. 293.
[7] Above. No. 4. [8] See foot-note (No. 6) Table I., p. 293. [9] See foot-note (No. 7) Table I., p. 293.
[10] 'Experimental Investigations on the Behaviour of the B. anthracis in Water.' *Proc. Roy. Soc.*, 1893, p. 245.

TABLE III.—*continued*

Investigators and Date of Experiments	Source of Organism Introduced into Water	Temperature at which Infected Water was Maintained	Foul Water	Ordinary Potable Water Unsterilised	Ordinary Potable Water Sterilised	Distilled Water	Mineral Water	Sea-water or Concentrated Salt Solution	Remarks
Wolffhügel and Riedel [1] (1883)	Anthrax obtained from a gelatine culture. It is stated that only bacilli were present, but as the anthrax was taken from gelatine cultures, and not from animals, there is *no guarantee* that spores were not present.	35° C. 35° C. 16° C. 7°–10° C.	10 and upwards of 15 days. * Upwards of 15 days. † Upwards of 15 days. ‡ Upwards of 9 days. §	—	—	—	—	—	* Unfiltered Panke water, sterilised. Also same water diluted with 10 volumes of distilled water. † Filtered Panke water, sterilised. Same results also when diluted with 10 volumes of distilled water. ‡ Filtered Panke water, sterilised and diluted with an equal volume of distilled water. § Filtered Panke water, sterilised and diluted with 10 volumes of distilled water. The anthrax was very attenuated, for $\frac{1}{3}$ c.c. of water only succeeded in killing a mouse after $4\frac{1}{2}$ days.

[1] See foot-note (No. 8) Table I., p. 293.

TABLE IV.

Vitality of Bacillus Tuberculosis in Water

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Chantemesse and Widal [1]	—	—	—	—	50 to 70 days.*	—	—	—	*Sterilised Seine water.
Straus and Dubarry [2] (1889)	A needle-point from a glycerine jelly culture introduced into 10 c.c. of water.	20° C. 35° C. 38° C.	— — —	— — —	27 days.* 30 days.* 95 days.* ‡	24 days.† 25 days.† 115 days.†	—	—	*Sterilised Ourcq water. †Sterilised distilled water. ‡Some of this water was inoculated into a guineapig. The animal was killed after 2 months and only bacilli were found in an abscess at the point of inoculation; no general tubercular symptoms were found. The authors state that doubtless by long residence in the water the virulence of the bacilli had become attenuated.

[1] Quoted in Straus and Dubarry's Paper, *loc. cit.*

[2] *Loc. cit.*

TABLE V.

Vitality of Staphylococcus pyogenes-aureus in Water

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Braem [1] (1889) .	—	—	—	—	—	25–50 days.	—	—	—
Ferrari [2] (1888) .	Two needle-loops from a fresh bouillon culture of the Staphylococcus introduced into 10 c.c. of water.	16°–18° C.	—	—	—	Over 5 days to several weeks.	—	—	—
Meade Bolton [3] (1886).	—	20° C. 35° C.	Over 340 days.‡ From 2–5 days; none found on the 10th day.‡	—	Up to 20 days; none found on the 30th day.* None found after 5 days.*	Up to 20 days; none found on the 30th day.† None found after 5 days.†	—	—	* Sterilised Berlin tap-water. † Distilled water. ‡ Grossly contaminated well-water, sterilised.
Slater [4] (1893) .	Agar culture at 37°C., 18–48 hours old, inoculated into sterile distilled or sterile soda-water	Ordinary temperature	—	—	—	—	2 days; dead on the 6th day.* 4 days; dead on the 5th day.† 11 days; dead on the 12th day.‡	—	* Simple aërated non-sterile water. † Sterile soda-water. ‡ Sterile soda-water, not aërated.
Straus and Dubarry [5] (1889).	—	20° C. 35° C.	—	—	19 to 21 days.* 15 days.*	4 to 9 days.† 13 days.†	—	—	* Sterilised Ourcq water. † Sterilised distilled water.

[1] *Loc. cit.*
[2] 'Ueber das Verhalten von pathogenen Mikro-organismen in den subcutan-einzuspritzenden Flüssigkeiten.' *Centralblatt für Bakteriologie*, vol. iv., 1888, p. 744.
[3] *Loc. cit.* [4] *Loc. cit.* [5] *Loc. cit.*

TABLE VI.

Vitality of Streptococcus pyogenes in Water

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Steriliesd	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry[1] (1889).	A needle-point from a gelatine culture introduced into 10 c.c. of water.	20° C.	—	—	14 days.* 15 days.†	8 to 10 days.‡	—	—	* Sterilised Ourcq water. † Sterilised Vanne water. ‡ Sterilised distilled water.

[1] *Loc. cit.*

TABLE VII.

Vitality of Streptococcus erysipelatis (Fehleisen) *in Water*

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Frankland, Percy[1] (1887)	Taken from a broth culture and diluted 75,000 times in the water under examination.	Rather under 20° C.	2 to 5 days.‡	—	5 days.†	Less than 1 hour.*	—	—	* Sterilised distilled water. † Sterilised London water (Thames). ‡ Sterilised London sewage.

[1] *Society of Chemical Industry, loc. cit.*

TABLE VIII.

Vitality of Micrococcus tetragenus in Water

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Hochstetter[1] (1887)	0·1 c.c. of a bouillon culture introduced into 50 c.c. of the water under examination.	10°–17° C.	—	—	8 to 18 days. None found on the 30th day.†	Upwards of 11 days. None found on the 18th day. ‡	4 to 8 days. None found on the 11th day.*	—	* Unsterilised Seltzer water. † Sterilised Berlin tap-water. ‡ Sterilised distilled water.
Meade Bolton[2] (1886).	—	20° C.	Upwards of 2 days. None found between the 4th and 6th day.†	—	Less than 4 days.‡	Less than 4 days.*	—	—	* Distilled water. † Grossly contaminated well-water, sterilised. ‡ Sterilised Berlin tap-water.
		35° C.	Upwards of 4 days. None found on the 6th day.†	—	Less than 4 days.‡	Less than 4 days.*			
Straus and Dubarry[3] (1889).	—	20° C.	—	—	19 days.*	19 days.†	—	—	* Sterilised Ourcq water. † Sterilised distilled water.

[1] *Loc. cit.* [2] *Loc. cit.* [3] *Loc. cit.*

TABLE IX.

Vitality of Bacillus of Mouse Septicæmia in Water (Bacillus murisepticus)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry [1] (1889).	—	20° C.	—	—	20 days.°	19 days.†	—	—	° Sterilised Ourcq water. † Sterilised distilled water.

[1] *Loc. cit.*

TABLE X.

Vitality of Rabbit Septicæmia in Water (Bacillus cuniculicida)

Investigator and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Hochstetter [1] (1887)	Bouillon culture, cultivated from 1–3 days at 30° C.	10°–18° C.	—	—	—	30 minutes to 2 days.†	30 minutes to 1 day.°	—	° Unsterilised Seltzer-water. † Sterilised distilled water. Pfeiffer (*loc. cit.*) states that in his experiments this bacillus died in sterilised well-water 'after a short time.'

[1] *Loc. cit.*

TABLE XI.

Vitality of the Bacillus of Fowl Cholera in Water (B. choleræ gallinarum). See p. 329.

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry [1] (1889).	One needle-point from a gelatine culture introduced into 10 c.c. of the water under examination.	20° C. 35° C.	— —	— —	2 days. * 30 days.*	0 † 8 days. †			* Sterilised Ourcq water. † Sterilised distilled water.

[1] *Loc. cit.*

TABLE XII.

Vitality of the Bacillus of Swine Plague in Water (Rothlauf, Rouget du Porc)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry [1] (1889).	Traces of the culture inoculated into 10 c.c. of water under examination.	20° C.	—	—	17 days.*	34 days.†	—	—	* Sterilised Ourcq water. † Sterillsed distilled water.

Loc. cit.

TABLE XIII.

Vitality of the Glanders Bacillus in Water (B. mallei)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry[1] (1889).	A needle-point from a potato culture introduced into 10 c.c. of the water under examination.	20° C. 25° C.	— —	— —	28 days.* 50 days.‡	19 days.† 57 days.†	—	—	* Sterilised Ourcq water. † Sterilised distilled water. ‡ Vanne water sterilised.

[1] *Loc. cit.*

TABLE XIV.

Vitality of Friedländer's Bacillus in Water (B. pneumoniæ)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Straus and Dubarry[1] (1889).	A needle-point from a gelatine culture introduced into 10 c.c. of the water under examination.	20° C.	—	—	4 to 7 days. *	8 days. †	—	—	* Sterilised Ourcq water. † Sterilised distilled water.

[1] *Loc. cit.*

TABLE XV.

Vitality of the Finkler-Prior Bacillus in Water

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Frankland, Percy[1] (1886).	One or more needle-loops of the culture were inoculated into about 50 c.c. of sterilised distilled water; from this attenuation a certain number of drops were introduced into the water under examination.	18°–20° C. 35° C.	None found on the 2nd day.* Ditto.*	—	None found on the 2nd day.†† Ditto.†‡	1 day.§	—	—	* Sterilised London sewage. † Sterilised London tap-water (Thames). ‡ Sterilised deep-well water. § Sterilised distilled water.
Hochstetter[2] (1887)	Taken from a potato culture.	18°–20° C.	—	—	2 days.*	15 minutes to 2 hours. None found after 4 hours.‡	4 hours.†	—	* Sterilised Berlin tap-water. † Unsterilised Seltzer-water. ‡ Sterilised distilled water.
Slater[3] (1893)	Agar culture 37° C., 48 hours old, inoculated into sterile distilled or sterile soda-water, 1·5–2 c.c. of which were employed for each inoculation.	Ordinary temperature.	—	—	—	5 and 24 hours; dead after 6 hours and 2 days respectively.*	Found extinct at the end of 2 hours.† 55 minutes; found dead at the end of 1 hour.‡ 4 & 10 days, but dead on the 6th and 12th days respectively§	—	* Sterilised distilled water. Results of 2 separate experiments. † Simple aërated non-sterile water. In another experiment it was found alive after 30 minutes, but dead at the end of 1 hour. ‡ Sterile soda-water. § Sterile soda-water, non-aërated. These are the results of 2 separate experiments.

[1] 'On the Multiplication of Micro-organisms,' *Proc. Roy. Soc.* 1886, p. 526. [2] *Loc. cit.* [3] *Loc. cit.*

Vitality of Bacillus pyocyaneus in Water (Green Pus)

Investigators and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Frankland, Percy[1] (1886).	See Table XV.	18°–20° C. 35° C.	Innumerable after 18 days.* Ditto.	— —	Innumerable after 8 days.† Ditto.	Upwards of 53 days.‡ Ditto.	—	—	* Sterilised London sewage. † Sterilised deep-well water. ‡ Sterilised distilled water.
Straus and Dubarry[2] (1889).	Traces of the culture introduced into 10 c.c. of the water under examination.	20° C.	—	—	20 days.* 73 days.†	20–73 days. ‡	—	—	* Sterilised Ourcq water. † Sterilised Vanne water. ‡ Sterilised distilled water. It is not stated at what temperature these distilled water experiments were made.

[1] *Loc. cit., Proc. Roy. Soc.* [2] *Loc. cit.*

TABLE XVII.

Vitality of Aspergillus flavescens in Water

Investigator and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Hochstetter[1] (1887)	Taken from an agar-agar culture kept for 3 days in the incubator and containing spores. This culture was obtained by inoculations from the kidney of a rabbit which had died 4 days after being infected with the Aspergillus flavescens.	12°–17° C.	—	—	56 days.*	56 days.‡	—	56 days.†	* Sterilised Berlin tap-water. † Unsterilised Seltzer-water. ‡ Sterilised distilled water.

[1] *Loc. cit.*

TABLE XVIII.

Vitality of the Tetanus Bacillus in Water. See p. 330.

Investigator and Date of Experiments	Source of Organism	Temperature at which Water was Maintained	Foul Water	Ordinary Potable Water, Unsterilised	Ordinary Potable Water, Sterilised	Distilled Water	Mineral Waters	Sea-water or Concentrated Salt Solution	Remarks
Schwarz, Rodolfo [1] (1890).	Virulent cultures containing spores; 10 drops of such a culture were introduced into flasks containing respectively 50 c.c. of the water under observation. To test the *initial* virulence of this dilution, from time to time 2 c.c. were inoculated into rabbits, and the latter invariably died of typical tetanus in 2 to 3 days.	Temperature of the laboratory varying between 4° and 18° C.	Stagnant water obtained from a moat, unsterilised.†† Very foul water, unsterilised.‡‡	Well-water.¶ Spring water.¶ Water from a cistern in the University at Bologna.¶ Water from the River Reno shortly before it leaves the city of Bologna.§§	Well-water.°† Spring water.°‡	Sterilised.*§ Unsterilised.¶	—	Sea-water obtained from Venice, sterilised.*‖ Unsterilised.*°	* All these waters were sterilised by heating to 100° C. for 1 hour. † Tested by direct inoculation of the water into rabbits, and found to be unabated in virulence on the 68th day. ‡ Ditto on the 51st day. § Ditto on the 136th day. ‖ Ditto on the 84th day. ¶ In the case of the *unsterilised* well-water, spring water, distilled water, and cistern-water, the tetanus organisms retained their full virulence during the first month; after that time, from the 30th to the 40th day, the virus showed signs of attenuation, and failed altogether in some cases to kill rabbits, whilst in others its effect was greatly retarded. This period of partial or complete attenuation did not last, says Schwarz, more than about a month, for at the end of that time portions of these same samples of water on being inoculated into animals under precisely similar conditions were found to have recovered their initial virulence. Thus the spring water had recovered its virulence on the 55th day from its original infection with tetanus; the well-water on the 70th day; the distilled water on the 79th day; the cistern-water on the 84th day. From this time on the virulence appeared to remain unabated; the spring water, which was tested on the 120th day, induced the death of an animal after 5 days with the typical symptoms of tetanus. *° Although originally virulent, the tetanus organism became attenuated within the first 10 days, and failed to kill rabbits, or was so delayed in its action that 54 days elapsed before the animal finally succumbed to tetanus. Animals inoculated with this water after 77 and 111 days respectively since its first infection with tetanus germs suffered no evil results whatever beyond becoming slightly thinner. It was proved that the organisms were not dead, for on adding ½ c.c. of this water in which the tetanus microbes had resided for 114 days to a tube of gelatine, and placing it in the incubator at 35° C., a virulent culture of sporiferous bacilli was obtained, a few drops of which destroyed a rabbit in 24 hours. †† Partial attenuation observed of the virus on the 36th day; complete attenuation on the 46th day. ‡‡ On the 50th day *no* attenuation of the virus was perceptible. This was a very foul water, rich in organic matter, and Schwarz states that it would appear that the presence of organic matter favoured the vitality of the tetanus organism. §§ Attenuation of the virus was observed between the 16th and 32nd day.

[1] 'Ricerche sulla vitalità del virus tetanico nelle acque' [illegible]

Schönwerth ('Ueber die Möglichkeit einer von Brunnenwasser ausgehenden Hühnercholera-Epizootie,' *Archiv f. Hygiene*, vol. xv. 1892, pp. 60–106) has investigated the virulence of the B. choleræ gallinarum (p. 324) in water by infecting certain wells, the temperature of which was 6·5°–7·9° C., with broth-cultures of the organism, and subsequently using it in feeding fowls and pigeons. Although these animals were fed for 16–20 days with this water, none of them developed any symptoms of chicken cholera. The same results were obtained in two distinct series of investigations. In another experiment the conditions were slightly varied, the infected water being rendered just alkaline with soda before being given to one of the fowls. This bird died in fourteen days of typical fowl-cholera. In another investigation, instead of infecting the well-water with broth-cultures, the bacilli were introduced without adding any culture material, but the same negative results were obtained. In another experiment the water was infected with the blood and juices of organs of birds which had died of chicken-cholera, but no pathogenic effect resulted. In order to test the actual virulence of the water some of the birds were inoculated with increasing doses (1–8 c.c.) of the water in each case; it was in this manner ascertained, judging by the length of time which elapsed between the infection and death of the bird, that in one case the virulence of the water infected with broth-cultures lasted 540 hours, in another 260 hours, whilst in the water infected with bacilli without broth, and in which a larger number were introduced, the virulence disappeared in 144 hours. In the case of the water into which the blood and juices of infected birds were introduced the virulence only disappeared after 220 hours, in spite of a much smaller number being employed. The author is of opinion,

therefore, that the bacilli introduced directly from the body of an animal are more vigorous and virulent than those taken from cultures; also that the simultaneous introduction of culture material with the bacilli serves to prolong the virulence of the latter.

The experiments on the behaviour of the sporiferous tetanus bacillus are amongst the most interesting which have been made in connection with the vitality of pathogenic bacteria in water. In some respects the results resemble those obtained by one of us on anthrax spores —thus, in the practically indefinite virulence in sterile waters, and the degeneration in unsterile ones. Altogether unique, however, is the recrudescence of the virulence exhibited by the tetanus bacilli, which is an observation of such fundamental importance as to demand further investigation.

Schwarz explains this attenuation of the tetanus organisms in the unsterilised waters as a consequence of the multiplication of the ordinary water bacteria present. He examined by means of plate culture the behaviour of the latter, and found that from the day of the introduction of the tetanus bacilli until the period when the attenuation of the latter was observed these water forms multiplied steadily, until the point was reached to which we have so often referred when their numbers again began to diminish. Schwarz throws out the suggestion that the attenuation of the tetanus organism may not only be due to the extensive multiplication of the water bacteria, stimulated by the culture-material which was introduced when the waters were first infected with tetanus, but may also depend upon the particular varieties of water microbes present; and that the recovery of their virulence by the former may be due to the subordination of the latter when their diminution became marked.

In order to show that this attenuation of the tetanus virus in unsterilised waters is due to the presence of other bacteria, Schwarz inoculated sterilised sea-water, not with a pure culture of tetanus, but, on the contrary, with an impure though virulent cultivation. Instead of the tetanus remaining for an indefinite length of time in a virulent condition, as had previously been observed in the case of sterilised sea-water, it was found to have become on the twentieth day already so attenuated, that on inoculating animals in the usual manner with some of the water their death only took place after a lapse of twenty-one days from the date of inoculation, and unaccompanied by any typical tetanic symptoms excepting extreme emaciation.

It will be seen from a careful study of the numerous investigations which have been made on the behaviour of pathogenic organisms in water what enormous differences exist in their capacity for living in this medium; that whereas some varieties are destroyed in a few hours, some will live and flourish for many months. Moreover, it is obvious how greatly in some cases the character of the water influences the vitality of the micro-organisms, a point which is especially brought out in Trenkmann's experiments on the cholera bacillus; and it is of particular interest to note how in the case of unsterilised waters the ordinary water bacteria are able to overwhelm and suppress those foreign pathogenic forms which have been introduced. Kraus's experiments are particularly instructive in this respect, for they exhibit the multiplication which takes place in the ordinary water bacteria, and the simultaneous disappearance which ensues of the pathogenic organisms introduced. The experiments on anthrax demonstrate conclusively the immensely greater vital powers possessed by the spore over the bacillar form

when introduced into water, and the necessity of differentiating between these two forms in carrying out such researches. Moreover, it cannot be too rigidly insisted upon that every precaution must be taken to avoid the introduction with the organism of any appreciable quantity of culture-material into the experimental waters. It is apparent how entirely the character of the water may be modified by a neglect of this precaution, and that a water originally incapable of supporting a pathogenic organism in question may be transformed into a suitable medium in which it will not only live but multiply. Unfortunately, many investigators have ignored this possibility, and hence we sometimes find such conflicting information recorded of the behaviour of one and the same organism in water.

CHAPTER IX.

THE ACTION OF LIGHT ON MICRO-ORGANISMS IN WATER AND CULTURE MEDIA

The above subject, although at first sight somewhat outside the application of bacteriological science which we have so far been considering, is one of such immense importance in connection with the vital phenomena of bacteria, and is indissolubly associated with so many of the problems with which the investigator is confronted, that a knowledge of what has been already achieved in this direction is essential to those who purpose taking up the study of micro-organisms in water. Indeed, some of the more recent experiments have a direct bearing upon the questions we have been discussing, and whilst, therefore, giving a general survey of the researches on light which have so far been made, a more particular account will be found of those investigations which are concerned with the behaviour of micro-organisms in water when exposed to light.

To Downes and Blunt[1] belongs the credit, not only of having first demonstrated the bactericidal effect of light in a memoir published in the Proceedings of the Royal Society in 1877, but in having at once so perfectly indicated almost all the factors connected with this action that the further investigations which have been carried out on this subject during the past fifteen years

[1] 'Researches on the Effect of Light upon Bacteria and other Organisms,' *Proc. Roy. Soc.* vol. xxvi. No. 184, 1877, Dec. 6.

have been little more than confirmations or additions of detail to their classical work. These investigators used sterilised Pasteur solutions, into which either a minute quantity of a liquid swarming with bacteria was introduced, or infection was secured by exposing the tubes for a short time to the air. These infected test-tubes were allowed to stand near a window with a south-east aspect day and night for days, weeks, and even months, direct sunlight reaching them for only a few hours daily. As a control, some of these tubes were carefully covered up in lead paper in order to expose them to the same conditions as the others, the light only being excluded. According as the solutions became turbid or remained clear it was inferred that the organisms had or had not grown, but microscopical examination was also frequently resorted to in order to determine this point more certainly. As a result of their observations, they stated that under favourable conditions exposure to light entirely prevented the development of these bacteria, whilst under less favourable conditions their growth was only thereby retarded ; moreover, that the direct rays of the sun were found to act most prejudicially, although diffused daylight had also a damaging effect. Experiments were also made and published later[1] to ascertain whether any particular, and if so what, rays of the spectrum acted more inimically than the rest upon bacteria, and it was found that bacteria exposed to the red and orange-red rays had their development delayed, whilst the blue and violet rays entirely prevented their growth.

Downes and Blunt explain this action of light as due to the gradual process of oxidation which is induced by the sun's rays in the presence of oxygen, which process,

[1] 'On the Influence of Light upon Protoplasm,' *Proc. Roy. Soc.* vol. xxviii., 1878, p. 199.

they suggest, very prejudicially affects the bacterial protoplasm itself; for they also found that if spores were exposed to light in a vacuum their power of germination was not impaired, and further that the nutritive value of the culture-liquids which they employed was not injured by insolation. Again, they showed that insolation can destroy bacterial life in the entire absence of culture-material altogether. These investigators, moreover, extended their researches to the action of light on soluble ferments, and showed that the invertase of yeast is destroyed by insolation in the presence of air, but not *in vacuo*. Finally, they found in a number of very striking experiments that bacteria are much more resistant to insolation when immersed in water than when surrounded by any other medium, a result which is obviously of great practical importance, and to which we shall have to refer again.

Tyndall [1] was led to test these remarkable investigations by taking flasks containing sterile solutions of cucumbers and mangel-wurzel to Switzerland, where he infected them with air on the Alps; but although exposed during varying periods of time to sunlight, no difference in the behaviour of the organisms could be detected, in all cases growths making their appearance. In the following year Tyndall [2] again made some experiments on the Alps, this time, however, using more nourishing culture media, and whilst some flasks were infected with putrefying animal and vegetable infusions, into others water from a stream was introduced. In these investigations it was found that the sun's rays had a decidedly damaging effect upon the bacteria present, but that

[1] 'Note on the Influence Exercised by Light on Organic Infusions,' *Proc. Roy. Soc.* vol. xxviii., 1878.

[2] 'On the Arrestation of Infusorial Life,' *Nature*, Sept. 15, 1881, vol. xxiv. p. 466.

their destruction was not accomplished, for when removed from the sunlight the hitherto clear liquid became gradually turbid. In consequence of the contradictory results obtained by Downes & Blunt and Tyndall respectively, Jamieson [1] made a series of investigations in Melbourne, using Cohn's solution. This worker found that the temperature of the solutions when exposed to direct sunlight rose to as high as 51° C., and that this was by no means the highest point reached. He, therefore, attributed the action of light not to the sun's rays, but to the high temperature which is thus produced in the solutions employed, a temperature which he declared to be sufficient in itself to destroy the bacteria present. He further states that when exposed during April to the sun's rays, when the temperature of the solutions did not rise beyond 36° C., the bacteria present were not destroyed.

Downes and Blunt [2] replied to this criticism by saying that, although some varieties of bacteria might doubtless be destroyed through this high temperature, yet others, and especially their spores, could certainly successfully withstand a considerably higher temperature.

It was evident that conflicting results would be obtained as long as *mixtures* of micro-organisms were the subject of investigation, and Duclaux,[3] therefore, attacked the question by employing pure cultivations of bacteria. The particular form chosen was a fermenting bacillus, Tyrothrix Scaber, obtained by this investigator from milk, and was made use of for this purpose

[1] 'The Influence of Light on the Development of Bacteria,' *Nature*, vol. xxvi., 1882, No. 663.

[2] 'On the Action of Sunlight on Micro-organisms, with Demonstration of the Influence of Diffused Light,' *Proc. Roy. Soc.* vol. xl. No. 242, 1886.

[3] 'Influence de la Lumière du Soleil sur la Vitalité des Germes de Microbes,' *Comptes rendus hebdomadaires des Séances de l'Académie des Sciences de Paris*, 1885, tom. c., p. 119, 12 Janvier.

in the act of spore-formation. This bacillus was inoculated into both milk and broth, and from each cultivation a small drop was introduced into separate sterile empty flasks, in which the drops were allowed to evaporate. The flasks, each of which thus contained the dry residue of a drop, were then exposed to sunlight for definite lengths of time, after which a small quantity of sterile bouillon or some other suitable culture material was added to the various flasks, the latter being then placed in the incubator. After fourteen days' exposure in the case of the milk-cultures the sunlight had produced no effect; after one month's insolation the development of the spores had been delayed; after two months' insolation two out of four of the flasks remained sterile. Spores similarly treated, but shielded from light, were found capable of development even after three years. On the other hand, the spores obtained from the bouillon (Liebig extract) cultures were far more quickly affected by exposure to sunlight than those obtained from milk. Thus, after fifteen days' insolation, out of three flasks one remained sterile; after one month's exposure two out of the three showed no growth; and at the end of two months all three flasks were sterile.

Duclaux therefore concludes that the degree of resistance to the action of light possessed by bacteria varies, not only according to the species, but also, in one and the same species, according to the nature of the culture medium from which they are obtained, as well as the intensity of the light to which they are exposed.

In a second investigation Duclaux[1] used six varieties of micrococci, and took precautions to prevent the tem-

[1] 'Influence de la Lumière du Soleil sur la Vitalité de Micrococcus,' *Compt. rend.*, 5 Août, 1885, tome ci.

perature from rising beyond 40° C. These cocci forms were introduced into neutralised veal-broth, and it was found that when preserved from the action of light, or exposed only to diffused light, they remained alive at least a year. An exposure to the sun in the spring and early summer (May 4 to June 13) could not be borne by them for longer than forty days, whilst in July fifteen days' insolation was sufficient for their destruction. The cocci of 'Clou de Biskra' and 'Pemphigus,' after being preserved in a dried condition in the dark for five or six months, succumbed after eight, three, and even two days' insolation in July. The micrococci, therefore, were much more rapidly destroyed by light than bacilli, and they were also found to be less refractory in a dry than in a moist state. According to Duclaux, therefore, the temperature has nothing to do with the action of light. Almost simultaneously with these experiments by Duclaux, Arloing[1] examined the effect of light on the anthrax bacillus, and this is the first instance of a pathogenic bacillar form being selected for investigation. The culture medium employed was chicken-broth, and the experiments were carried out in a dark room, a large gas-lamp being the only source of illumination. Test-tubes containing sterile broth were inoculated either with the spores or bacilli of anthrax, and placed in an incubator. The latter was divided into two chambers, the one being dark, whilst the other was lined with white paper, and admitted light from the powerful gas-burner in question. It was found that the anthrax bacilli flourished equally well whether preserved in the dark or exposed to diffused gas light, but that when exposed to gas-light

[1] 'Influence de la Lumière sur la Végétation et les Propriétés pathogènes du Bacillus Anthracis,' *Compt. rendus hebd. des Séances de l'Académie des Sciences*, 9 Fév., 1885, tome c., p. 378.

concentrated by means of a lens their growth was impeded. The blue and violet rays of this gas-light were found to act prejudicially upon the development of both the spores and bacilli, and also impeded the sporulation of the latter.

In subsequent experiments on anthrax Arloing[1] substituted the light of the sun for that of the gas-burner. Chicken-broth was again employed as the culture material in which the bacilli and spores were exposed, and as soon as the day's insolation was over the tubes were placed in a refrigerator to prevent any multiplication taking place in the interval. Finally, when the period of insolation was completed, in order to ascertain whether the anthrax was still alive or not, the tubes were placed in an incubator and maintained at a suitable temperature. Arloing found that insolation for two hours, when the temperature registered was between 35° and 39° C., was sufficient to entirely destroy the spores of anthrax. If, however, the exposure was less than two hours, from 1 to $1\frac{1}{2}$ hour, the development of the bacilli took place only after 16, 18, 30, 72, or even 96 hours, whilst in those tubes kept in the dark incubator at as nearly as possible the same temperature as those in the sunlight the bacilli developed already in from 8 to 12 hours. If tubes containing spores were kept in the incubator for from 24 to 48 hours at a suitable temperature for the development of the bacilli, and were then exposed to the light, when they contained presumably no spores, it was found that 2 hours' insolation was not sufficient to destroy the bacilli, but that from 26 to 30 hours were necessary, even in the presence of a temperature of from 30°

[1] 'Influence du Soleil sur la Végétabilité de Spores du Bacillus Anthracis,' *Compt. rendus*, 24 Août, 1885, vol. ci. p. 511. Also see a further note published in the same volume, 31 Août, p. 535.

to 36° C. It should be mentioned that, in order to obviate the formation of spores, the tubes were, during the period over which the insolation extended, always placed at night in a dark refrigerator. According to Arloing, therefore, the spores are less capable of withstanding the effect of light than the bacilli, an observation which is out of harmony with the commonly accepted views concerning the greater powers of endurance possessed by the former. In connection with the individual characteristics of micro-organisms observed in one and the same species which offer such a puzzling phenomenon in the behaviour of bacteria, the following observations of Arloing are of much interest, as being an illustration of the manner in which the previous history of a micro-organism affects its subsequent behaviour.

It was observed that if a cultivation be insolated for such a period of time which, whilst damaging it, did not destroy it, the next generation derived from this culture exhibited a tardiness in vegetation according as the period of insolation to which the mother-culture had been exposed was long or short. For example, if the latter had been exposed to light for from 4 to 8 hours, the development of the next generation was delayed for from 20 to 24 hours, whilst if the original culture was exposed to from 15 to 20 hours' insolation, its progeny were unable to exhibit any signs of growth for from 36 to 40 hours. Nor was this all, for an exposure of from 9 to 10 hours sufficed to kill a generation the mother-culture of which had survived 25 hours' exposure to light. Arloing also ascertained that these weakened cultures were less virulent when inoculated into animals; in fact, that after about 30 hours' insolation such anthrax cultures were converted into a sort of vaccine, for guinea-pigs inoculated with

them not only remained alive, but acquired a more or less pronounced degree of immunity.

Nocard[1] objected to Arloing's conclusions as to the greater susceptibility to light of the spores than the bacilli of anthrax, by suggesting that during the exposure of the spores they had developed into bacilli, and that the light had acted on the latter, and not on the spores, as maintained by Arloing.

Straus,[2] in order to bring experimental evidence to support this assertion of Nocard's, inoculated anthrax spores into sterilised distilled water, whilst, as a control, a series of inoculations was also made into sterile bouillon; both sets of tubes were exposed to nine hours' insolation. The result of these comparative experiments was that all the spores in the bouillon were found to be dead, whilst those immersed in distilled water yielded growths when inoculated into sterile bouillon. Straus explains this difference by observing that whereas in broth the spores were able to germinate into bacilli in spite of the exposure to light in the distilled water, which does not afford a suitable medium for the germination of the spores, they remained, therefore, in the condition of spores, and were thus able to withstand the action of light.

Arloing[3] soon after published another memoir, controverting Straus's conclusions. In this paper he refers to experiments in which he distributed anthrax spores in broth contained in vessels placed in contact with ice

[1] *Recueil de Médecine vétérinaire*, 1885.

[2] 'Charbon de l'Homme et des Animaux,' *Société de Biologie*, 1886, p. 473.

[3] 'Les Spores du Bacillus Anthracis sont réellement tuées par la Lumière solaire,' *Compt. rendus*, 7 Mars, 1887, vol. civ. p. 701. See also another paper published by Arloing in reply to Nocard: 'Influence de la Lumière blanche et de ses Rayons constituants sur le Développement et les Propriétés du Bacillus Anthracis,' *Archives de Physiologie normale et pathologique*, 1886, vol. vii., No. 3, pp. 209–235.

during their exposure to light, the temperature being thereby kept below 4° C. By the low temperature thus secured Arloing considered that he had effectually banished the possibility of the spores germinating into bacilli, and under these conditions again he was able to demonstrate the complete destruction of the anthrax in five hours. Arloing, however, also found that a longer period of insolation was necessary for the destruction of anthrax spores when the latter were placed in water. A further confirmation of these experimental results, but with an entirely different explanation, was furnished in an exceedingly interesting and suggestive paper by Roux, to which we shall presently refer.

Meanwhile some fresh observations of Arloing must be recorded concerning the conditions under which light acts upon bacteria. In one of his memoirs (see note 1, p. 339) already referred to, the observation is made that if an obstacle be placed between the source of light and the object of experiment in the shape of a layer of distilled water a few centimetres thick, the bacteria thus exposed will remain almost as little affected as if they were kept in darkness. This result is confirmed by a number of experiments made by this investigator, and recorded in another memoir published in the *Archives de Physiologie* (see note 3, p. 341). Here it is stated that a layer of distilled water two centimetres thick placed between the sun's rays and the experimental vessels will almost completely nullify the deleterious effect of the light, but that not all liquids are possessed of this protecting power, for out of three experiments in which a solution of alum was employed in the place of distilled water, in two the spores were destroyed as usual, whilst in the other case their development was delayed for twenty-four hours. Arloing does not seek to explain these most remarkable results,

and it will be seen later that as regards a layer of water, even $1\frac{1}{2}$ foot in depth, intervening between the source of insolation and the object insolated, Buchner found that it had no appreciable effect whatsoever, at any rate in the case of the micro-organisms with which he experimented, viz., typhoid and cholera, Bacillus Coli-communis, Bacillus pyocyaneus, and Bacillus prodigiosus.

The extraordinary results obtained by Arloing, pointing to the more refactory nature of the bacillar than the spore forms of anthrax towards insolation, led Roux[1] to submit the whole question to an experimental reinvestigation. The method adopted by Roux was as follows:—

A drop of blood containing anthrax bacilli was inoculated into the aqueous humour obtained from the eye of an ox. This was cultivated for ten days, and finally all bacillar forms destroyed by an exposure of the liquid for ten minutes to a temperature of 70° C. One drop of this culture was then deposited on the bottom of a sterilised test-tube, the mouth of which was subsequently sealed up in the flame of the blow-pipe. Fine capillary glass tubes were simultaneously completely filled with the same culture fluid and their ends sealed in the flame. In the test-tubes, therefore, about 20 c.c. of air were present, whilst the capillary tubes contained no air at all. These tubes were all exposed during the same length of time to a July sun, the temperature inside the tubes not exceeding 39° C., even on the hottest days. At the end of the insolation one of the test-tubes and one of the capillary tubes were opened, and in the case of the former sterile bouillon was added to the tube, whilst the whole contents of the

[1] 'De l'Action de la Lumière et de l'Air,' *Annales de l'Institut Pasteur*, vol. i., 1887, p. 445.

capillary tube was transferred to a test tube containing sterile bouillon; both of these were then removed to the incubator and cultivated at 37° C. It was found that the spores in those tubes in which air was present had been destroyed according to the length of time they had been insolated, the period varying between 29, 30, and in one case 54 hours. On the other hand, the spores which had been insolated in the liquid sealed up in the capillary tubes (therefore in the absence of air) were found capable of germination even after 83 hours' exposure to the sun. In these experiments Roux took the special precaution of exposing the spores in the very medium in which they had been produced, precluding, therefore, the possibility of their germination into bacilli in this exhausted material. In other experiments bouillon[1] infected with anthrax spores was insolated, in some of the tubes air was excluded, whilst in others air was allowed to gain access. On the completion of the insolation both the aërobic and anaërobic tubes were placed in the incubator; in the case of the latter the contents were poured into an empty vessel to which air had access. It was found that whilst the spores which had been insolated in the absence of air were able to germinate even in the same bouillon in which they had been insolated, those spores which had been insolated in the presence of air were unable to germinate. Thus, in Roux's experiments the spores of anthrax were not destroyed with the same promptitude as in Arloing's. On the other hand we are introduced to a fresh problem, which had already, however, received considerable attention from Downes and Blunt, *i.e.*, the behaviour of insolated spores in the presence or absence of air. Roux shows very clearly that they

[1] Veal-broth, slightly alkaline and very faint in colour, consisting of 1 part of meat to 2 parts of water.

are far less injured by the action of light in the *absence* of air, and in order to further elucidate this phenomenon, he conducted experiments to ascertain what was the effect of insolation on the culture material itself.

For this purpose flasks (A) containing sterile bouillon, and others (B) into which anthrax spores had been introduced, were exposed side by side to the sun's rays. At the end of each hour a flask of each series was withdrawn, and into the sterile one spores were introduced, and both flasks were then placed in the incubator. Usually after two hours' exposure the (B) or infected flasks were found incapable of yielding growths, whilst in the case of the (A) or sterile flasks, into which the spores were introduced just before being placed in the incubator, growths usually made their appearance. If, however, the insolation of the sterile flasks had been prolonged for three or four hours, the bouillon contained in them proved to be no longer a suitable culture medium, for in spite of the introduction of the anthrax spores no signs of growth were visible. Under the action of the sun's rays in the presence of air, the bouillon had, therefore, undergone some chemical change which rendered it unsuitable for the germination of the spores.

The *spores* themselves, however, had not been destroyed either by two or even by seven hours' insolation, for when they were removed from the bouillon in which they had been insolated but in which they had *not* germinated, and were introduced into bouillon which had not been exposed to light, germination followed. The germination was, however, retarded according to the length of time during which they had been insolated or had been in contact with the insolated bouillon. If, however, instead of sowing the sterile insolated bouillon

with spores, bacilli were introduced (a drop of blood from an animal dead of anthrax, for example), the latter developed freely, thus showing that by the exposure to light the bouillon had been only so far modified as to prevent the germination of the spores, but not so as to inhibit the multiplication of the bacilli.

These results afford an explanation of the fact observed by Arloing, which he attributed to the greater powers of resistance in the presence of light possessed by the bacilli over the spores of anthrax. Roux's experiments show that this depended upon the bacilli being capable of growing in the modified insolated bouillon, whilst the spores were unable to do so.

That the presence or absence of air during insolation has an important effect upon the vital activity of the spores of anthrax has been shown very conclusively in the preceding experiments. Roux further demonstrated this by showing that if sterile bouillon was placed in vacuous tubes or in an atmosphere of carbonic-acid gas and was then insolated for several hours, on being subsequently inoculated with anthrax spores, the latter were able to germinate. On the other hand, if the same bouillon was subsequently insolated in the presence of air, the spores were unable to germinate when introduced into it. Roux also found that when culture media, which through previous insolation in the presence of air had been rendered unsuitable for the germination of spores, were subsequently kept for a certain time in the dark or in diffused light their original nutritive qualities were restored.

These exceedingly interesting and important experiments of Roux, by showing that the greater insusceptibility towards light of the bacillar than the spore

forms of anthrax is apparent only, and not real, serve to explain what was before a most anomalous phenomenon with regard to these two forms of anthrax, and also exhibit a fresh factor in the mechanism attending the bactericidal action of light, viz., the alteration which the culture fluid may itself undergo during insolation in the presence of air. We should, however, at once point out that this alteration of the culture medium through insolation has not hitherto been confirmed by many observers; but to this point repeated reference will be made hereafter.

The bactericidal action of light was again attacked by Gaillard,[1] who under the direction of Arloing carried out a number of experiments with different varieties of micro-organisms, the *Bacillus fluorescens*, *Staphylococcus pyogenes aureus*, *Bacillus prodigiosus*, *Bacillus anthracis*, *Bacillus typhosus*, *Penicillium glaucum*, *Oidium albicans*, and the 'Rosahefe' (pink yeast). No very important fresh results are recorded, but it was found that sunlight acted prejudicially on the production of colours by the so-called pigment producing bacteria, that the sun's rays appeared to favour the development of several kinds of yeast[2] and moulds, and finally that the action of light was increased by the presence of air.

The Italians now took up the subject, and we find a paper by Pansini[3] in which he describes his experiments on various organisms, *B. prodigiosus*, *B. violaceus*, *B. pyocyaneus*, *B. anthracis*, cholera bacillus, *B. muri-*

[1] *De l'Influence de la Lumière sur les Micro-organismes*, Lyon, 1888. See also a Paper by Uffelmann, *Die hygienische Bedeutung des Sonnenlichtes*, 1889.

[2] In this connection it is worthy of note that Downes and Blunt found that the organisms which survived insolation in tubes filled with pure oxygen were torula-forms.

[3] 'Azione della Luce solare sui microrganismi,' *Rivista d'Igiene*, 1889.

septicus, and the *Staphylococcus pyogenes albus*. These investigations were carried out at Naples in the zoological station there and were pursued throughout the year, care being taken that in every case a control experiment was made with cultures preserved in the absence of light, contained in blackened glass vessels, and placed side by side with those exposed to the sunlight. The temperature never rose beyond 45° C., and the difference of temperature in the darkened and the insolated flasks respectively never varied more than one degree. In this manner the possibility of temperature being responsible for the effect produced was disposed of. Pansini exposed, first, various culture media recently inoculated with different microbes; secondly, well-developed cultivations; thirdly, hanging drop cultures of infected bouillon. The second of these methods, which consisted in exposing well-developed cultivations on gelatine or potatoes, is obviously unsatisfactory, inasmuch as by reason of the thickness and opacity of the growth the sun's rays could not penetrate equally to all parts of the cultivation.

In the first method, test-tubes plugged with cotton-wool, some blackened and others not, containing gelatine or slices of potato, were inoculated with various microbes and exposed to the sun's rays. Every half-hour one tube from each series was removed and placed in the incubator, to watch its further development. In this manner it was found that light invariably exercised a retarding influence on the development of the organisms, and this effect was the more rapid the more nearly normal the angle at which the rays fell on the surface of the culture material. The rate of destruction varied, however, according to the micro-organism and the medium in which it was present. The *Bacillus pyocyaneus* was found to resist light generally better

than the *B. prodigiosus*. The anthrax bacillus was destroyed in four or five hours when exposed on potatoes, and in six or seven on gelatine. Pansini further found that this effect was due to the action of light on the bacteria and not on the culture material, for if the latter after this exposure was re-inoculated it was found to have lost none of its nutritive capacity.[1]

The chief novelty of Pansini's experiments lies in the more exact information they give us as to the rapidity at which bacteria are destroyed by the agency of light.

For this purpose, drop cultures of the various organisms were prepared in the usual manner, and after they had been insolated for a definite length of time the cover-glass was carefully removed and placed in melted gelatine, and after thorough agitation in order to insure the equal distribution of the organisms as well as to effect their entire removal from the surface of the cover-glass, plates were poured in the usual manner and the resulting colonies counted. As an example of the results obtained the following experiment may be cited :—Drop cultures were prepared of a young culture of the *B. anthracis*,[2] and exposed to sunlight for periods of time varying from ten to seventy minutes, during which the temperature ranged between 32° and 40° C. From the cover glass kept in the dark 2,520 colonies were obtained on the following day on the plates poured, whilst none had appeared on the plates

[1] The writers have been unable to ascertain what were the particular microbes referred to by Pansini as having been re-inoculated on to the various insolated media and found capable of growing. It will be remembered that Roux found that the *bacilli* of anthrax were able to develop in insolated bouillon, but not the *spores*.

[2] There is no guarantee that spores were not present, as no special precautions were taken to exclude them.

prepared from the insolated cover-glass. On the third day the following figures were obtained :—

Cover-glass exposed	10	minutes	to the	sun,	360	colonies
„	„	20	„	„	130	„
„	„	30	„	„	4	„
„	„	40	„	„	3	„
„	„	50	„	„	4	„
„	„	60	„	„	5	„
„	„	70 and more		„	0	„

Thus the destruction is rapid in the first period of exposure, but the more resistant of the microbes take a considerably longer time to annihilate, illustrating once more the individual peculiarities inherent in members of one and the same cultivation.

Pansini further states, what was affirmed by Arloing but explained and contradicted by Roux, that the spores of anthrax are more susceptible to light than the bacilli. He mentions that in bouillon containing anthrax spores and exposed to sunlight he has found from thirty minutes to two hours sufficient to destroy them, whilst bacilli under precisely similar conditions required from one to two and a half hours' exposure before being killed. It should be noted that in Pansini's experiments all question of the culture medium being affected by the insolation is removed, inasmuch as the insolated drops containing the organism (whether spores or bacilli) were subsequently transferred to fresh and unexposed culture material, viz., gelatine, in which their survival or decease was put to the test by plate-cultures. We are of opinion, however, that in this method of testing there may lie a source of serious fallacy, inasmuch as the development of anthrax spores in the gelatine-peptone medium is within our own experience often so greatly retarded as to lead to the belief that the spores are dead unless a very prolonged incubation of the plates is resorted to. This may, perhaps, account for

the results with anthrax spores obtained by Pansini, possibly the real interpretation being that they had not developed on the gelatine plates when the incubation of the latter was discontinued.

One more table may be quoted, which deals with the behaviour of anthrax spores when dried on cover-glasses and exposed to the sun :—

Dried Anthrax Spores [1]

Cover-glass kept in the dark					1,015	colonies
„	exposed 30 minutes to light				396	„
„	„	1 hour	„		208	„
„	„	2 hours	„		48	„
„	„	3 „	„		30	„
„	„	4 „	„		34	„
„	„	5 „	„		8	„
„	„	6 „	„		3	„
„	„	7 „	„		3	„
„	„	8 hours and more to light			0	„

Thus Pansini remarks that the spores of anthrax in a dry state are more capable of resisting the action of light than in a moist condition ; in the latter case an exposure of from 30 minutes to 2 hours, as before mentioned, being found sufficient to destroy them.

Pansini was further able to confirm the observations made by Gaillard, that light acted deleteriously on the production of pigment by bacteria. As regards the attenuation of the virus of anthrax by the agency of light, Pansini was less successful in the results he obtained than Arloing in his experiments already referred to.

As regards the effect of light on pigment-producing organisms some interesting investigations made by

[1] The authors were unable to discover in what medium Pansini dried the spores, whether water, broth, or gelatine, &c. This is of importance, as will be seen later from the results obtained by Momont in his desiccation experiments with anthrax spores.

Laurent [1] on a bacillus originally found in water (*Bacille rouge de Kiel*, see Appendix, p. 442) must be here referred to. This bacillus was inoculated on to slices of potato which were then exposed during 1, 3, and 5 hours respectively to the direct rays of a July sun, after which they were placed in the incubator at 33° C. On the potato exposed to the sun for one hour, white colonies mixed with a small number of pink centres made their appearance. On the potato exposed for three hours, colourless colonies, with the exception of a few which were pale pink, developed; whilst it was found that five hours' insolation had entirely destroyed the bacilli on the third slice. The white colonies from both the slices were inoculated on to slices of potatoes and kept in the incubator at 33° C. Nearly all the colonies resulting from the white colonies of the slice insolated for one hour were pink, whereas, with but one or two exceptions, the colonies inoculated from the slice exposed for three hours were colourless, and after being transplanted for the third time on to potatoes no trace of colour in the growth remained. Laurent says that the light had so far modified the physiological character of the bacillus that a colourless race, perfectly uniform in its behaviour in this respect, had been obtained. As many as 32 successive colourless generations of this bacillus were obtained on slices of potato kept in the incubator at from 25°–35° C., but if kept at from 10°–25° C. the colour reappeared.

By exposing colourless cultures three months old to 56° and 63° C. for 5 minutes, a variety was obtained which remained colourless on potatoes at all temperatures. This new variety was very feeble and may be regarded as a degenerated form.

[1] Étude sur la Variabilité du Bacille rouge de Kiel,' *Annales de l'Institut Pasteur*, vol. iv., 1890, p. 478.

An exposure for three, four, or six hours to the sun in September and October was found also capable of modifying the pigment-producing powers of this bacillus, although the colour was not so uniformly removed, and a tendency to return to its original colour was noticeable.

Laurent explains the presence of pink colonies amongst the colourless centres as due to inequalities in the power of resistance possessed by individual bacilli, as well as to the uneven thickness of the growth in different parts of the potato-slice.

Some investigations were also made to determine whether the colour would be similarly affected in cultures kept in an atmosphere of hydrogen or carbonic acid, and it was found that it was only in the presence of air that insolation produced its full effect, and that a permanently colourless race of bacilli was obtained.

Laurent also found that all the rays of the spectrum acted prejudicially on the production of the pigment, but that the principal part in this respect was played by the most highly refrangible rays of the spectrum.

Still more recently, d'Arsonval and Charrin[1] have examined the action of sunshine on the pigment-producing power of the *B. pyocyaneus*. These investigators state that when this organism is exposed in a liquid (the nature of the medium is not mentioned, but it was presumably broth) for from 3 to 6 hours to sunshine, and is afterwards introduced (one drop being used) into agar-agar, and kept at 35° C., only colourless colonies make their appearance; if, however, it is exposed to the red rays only, the typical fluorescent green-coloured colonies are produced. If the exposure to sunshine is

[1] 'Influence des Agents atmosphériques, en particulier de la Lumière, du Froid, sur le Bacille pyocyanogène.' *Comptes rendus*, vol. cxviii. 1894, p. 151.

prolonged, the bacilli are completely destroyed, whilst they are unaffected if subjected during the same length of time to the red rays only.

In Santori's[1] experiments special attention is bestowed upon the part played by temperature during insolation. This investigator found (1) that the bactericidal action of light came into play even when unaccompanied by a high temperature; (2) that the red and violet rays of the spectrum have no specifically different action on bacteria; (3) that microbes are more refractory as regards light in the dry than in the moist state; (4) that no appreciable difference was noted in the relative powers of resistance possessed by the spores and bacilli of anthrax respectively; (5) that the action of the sun and of the electric light was greater according as the accompanying temperature was higher; (6) that the action of the electric light (900-candle power at a distance of 80 cm.) was distinctly weaker than that of the sun; and (7) that the virulence of the anthrax bacillus may be diminished through insolation, and be made to serve as vaccine.

Koch[2] states that the tubercle bacilli (see Appendix, p. 422) were destroyed in direct sunshine in from a few minutes to some hours, according to the thickness of the material in which they were suspended and exposed. Cultures of the tubercle bacillus were destroyed, even in diffused light, when placed close to the window, in from 5 to 7 days.

We now come to the researches of Russian investigators. Janowski[3] confined his observations to the

[1] *Bollettino dell' Accademia medica di Roma*, vol. xvi., 1889–90.

[2] 'Ueber bacteriologische Forschung:' Vortrag in der ersten allgem. Sitzung des x. internationalen medicinischen Congresses, 1890, quoted by Kitasato, *Zeitschrift für Hygiene*, vol. x. 1891, p. 285.

[3] 'Zur Biologie der Typhusbacillen,' *Centralblatt für Bakteriologie* vol. viii. 1890, pp 167, 193, 230, 262.

typhoid bacillus, and his first experiments were made to ascertain if this bacillus suffers any diminution in its vitality when exposed to *diffused* daylight. It was found that, when typhoid growths were exposed on sloping surfaces of gelatine in a cold room the temperature of which was about 12° R., growths were visible on the third day in the tubes protected from light with black paper, but that in the case of the exposed tubes nothing was observed until the fifth day. Again, when in the same room at 12° R., typhoid bacilli were introduced into sterile bouillon, rendered as clear as possible by the addition of four parts distilled water and 0·5 per cent. sodium chloride, turbidity did not commence in the case of the exposed flasks and tubes until from twenty-four to twenty-eight hours after inoculation, whereas in the protected flasks turbidity was noted sixteen to twenty hours after inoculation. These differences are not very great, but were maintained throughout a large number of experiments.

In direct sunlight the results were more striking. The temperature registered during the experiments never rose above 40° C. The same kind of broth was used as in the previous experiments, and the tubes, after inoculation, were exposed to direct sunlight on the roof from eight in the morning until seven in the evening; during the night they were placed in a refrigerator. In the protected tubes turbidity commenced after eight hours, whilst in the insolated tubes the liquid remained perfectly clear. After two days' insolation the tubes were placed in an incubator at 37° C., but no turbidity made its appearance. This result was invariably obtained throughout a large series of investigations. In order to ascertain whether the bouillon had undergone some chemical change during insolation unfavourable for the development of the

bacilli (see Roux's experiments), the following experiments were made:—A flask of bouillon infected with typhoid was insolated during twenty-two hours, at the end of which time the liquid was quite clear; by means of a sterilised pipette about 1 c.c. of this bouillon was transferred to another flask of sterile bouillon, which had not been insolated, and was placed in the incubator at 37° C. *No growths made their appearance*, showing that the typhoid bacillus had really been destroyed. The original flask of insolated bouillon infected with typhoid bacilli, which had remained quite clear, was re-inoculated with fresh typhoid bacilli and placed in the incubator at 37° C. *Turbidity quickly followed*, showing that the insolated bouillon had not undergone any change rendering it incapable of supporting the typhoid bacilli.

It will be remembered that in Roux's experiments it was found that the *bacilli* of anthrax could flourish perfectly in previously insolated bouillon, whilst the *spores* were unable to germinate except in bouillon insolated in the absence of air; hence, as Janowski's researches in this direction only extend to *bacilli*, we have really only a confirmation of the previous experiments made by Roux on anthrax bacilli.

Careful experiments showed that from four to ten hours' insolation sufficed to destroy the typhoid bacilli. Numerous investigations were also made to ascertain which were the rays of the spectrum which exercised this bactericidal effect; but as Janowski did not decompose the white light of the sun with a prism, but only separated out the rays by means of coloured solutions, his experiments in this direction are less precise than those of Geisler, to be mentioned below; he is, however, led to attribute the bactericidal action of light to the chemical rays of the spectrum, inasmuch as the sun-

light, after passage through a stratum of potassium dichromate solution, was without effect.

Geisler,[1] working in St. Petersburg, published a paper in February, 1892, on the action of light on bacteria, in which he endeavours to differentiate between the effects produced by the rays of the electric light and the rays of the sun respectively on typhoid bacilli. As culture material 10 per cent. gelatine-peptone was employed. A series of six test-tubes, containing gelatine, were inoculated with typhoid bacilli streaked over the surface. Two were placed in a dark cupboard, two were exposed to direct sunlight, and two were exposed to the light from a 1,000-candle electric arc lamp at a distance of 1 metre. It was found that in the latter case three hours' exposure produced a distinctly retarding effect upon the typhoid growth, but that after six hours this result was considerably more apparent. In the case of direct sunlight, on the other hand, two hours' insolation already produced a very markedly deleterious effect. The powerful electric light thus has, as would be anticipated, a less rapid bactericidal action than direct sunshine.

Experiments were made to ascertain if the action of light was affected by the accompanying rise in temperature, and for this purpose typhoid tube-cultures were exposed to the direct rays of the sun and electric lamp, after the latter had both been passed through a solution of alum, whereby the heat rays are absorbed, permitting the so-called light and chemical rays of the spectrum to pass unimpeded. The following results were obtained:—

In the control tubes kept in the dark during from two to three hours an abundant growth took place.

[1] 'Zur Frage über die Wirkung des Lichtes auf Bakterien,' *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 161.

In the tubes exposed to direct sunlight for from two to three hours, and to the electric light for six hours, in both cases passed through a solution of alum, only a slight growth was observed; whilst in the tubes exposed direct to the sun's rays and the electric light for from three to four and six hours respectively the least growth of all was observed. Thus the most deleterious effect was produced when the action of light was accompanied by heat, an observation which entirely accords with Santori's experience as regards the effect of temperature. Experiments were made to ascertain which rays were the responsible agents in the action of light, and for this purpose the rays of the spectrum were split up by means of a prism, and the gelatine tubes so arranged that only particular rays fell upon them. The exposure in the rays from the electric-light spectrum was continued over a period of from one to three to six hours, whilst two and a half hours' exposure was employed in the case of the solar spectrum.

Both series of experiments yielded the same result—viz., that the typhoid bacilli grew best in the red rays; in fact, no difference could be detected between their growth in these rays and in the dark cupboard. The development became more and more scanty in passing from the red to the yellow, green, and violet rays, whilst the least growth of all was observed in the ultra violet rays.

With reference to the electric light rays, it was found that one hour's exposure produced no sensible effect upon the growth, and that it was only after three hours' exposure that an appreciable result was obtained.

Geisler further made some experiments to ascertain if the culture medium was chemically changed by ex-

posure to light. It will be remembered that Roux stated that such an alteration took place in broth, whilst Pansini, when using gelatine, could trace no such effect, and Janowski stated that he could also find no such change produced in broth.

Two sterile gelatine tubes were exposed for from two to three hours to direct sunlight side by side with two into which typhoid bacilli had been introduced. Two control tubes were placed in a dark cupboard. The sterile insolated tubes were subsequently inoculated with typhoid bacilli, and were removed with the other tubes to a dark cupboard. The growth was markedly more feeble in the insolated tubes subsequently inoculated with typhoid bacilli than in the control tubes kept from the first in the dark cupboard; it was, however, somewhat better than in those tubes inoculated with the bacilli and insolated for the above period. Geisler is, therefore, of opinion that a distinctly unfavourable effect is produced upon gelatine during insolation, at any rate as far as its fitness for the cultivation of the typhoid bacillus is concerned.

Kotljar,[1] another Russian investigator, has made some experiments to ascertain which rays of the spectrum are mostly concerned in the bactericidal action of light. These were, however, not carried out by means of a spectroscope, but by surrounding the test tubes containing the bacteria under observation with different coloured films of gelatine. The culture media employed were agar-agar and potatoes. The results obtained as regards the particular organisms (non-pathogenic) investigated were not so striking as in the case of pathogenic varieties studied by other observers.

[1] 'Zur Frage über den Einfluss des Lichtes auf Bakterien,' Wratsch, 1892, Nos. 39 and 40. *Centralblatt für Bakteriologie*, vol. xii., 1892, p. 836.

Kotljar found, however, that the red rays appeared to favour the development of the micro-organisms, whilst the violet rays hindered their growth. This observer made by chance the interesting discovery that the violet rays, however, actually favoured the sporulation of the *B. pseudo-anthracis*.

We will next consider, for the sake of continuity, some experiments made by Buchner[1] on the action of light on the typhoid bacillus, although a memoir by Momont on the behaviour of anthrax bacilli during insolation comes first in chronological order of publication. The first paper by Buchner contains an account of some experiments on the effect of insolation on bacteria in water. As we shall take those investigations which deal especially with water separately into consideration, we will pass on to his second communication, in which nutritive agar-agar was employed as the culture medium. For this purpose agar-agar contained in test-tubes was rendered fluid, and then inoculated with pure cultivations of various bacteria and poured into Petri dishes. When the contents had solidified, a piece of black paper with letters of the alphabet cut out was fastened on to the under-side of the dish by means of an india-rubber band, and the dish was then exposed bottom upwards to direct sunlight for from one to one and a half hour, or during five hours to diffused light. After this exposure the dishes were incubated in a dark place. Already in twenty-four hours' time the letters of the alphabet employed appeared sharply defined on the culture material, for where the black paper screen had protected the bacteria underneath from the action of the sun's rays there

[1] 'Ueber den Einfluss des Lichtes auf Bakterien' (No. 1), *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 781. *Ibid.* (No. 2), *loc. cit.* vol. xii., 1892, p. 217.

colonies made their appearance, whilst that portion of the culture material which the sun's rays had reached through the cut-out letters was sterile, or nearly so. Buchner's paper is accompanied by a drawing copied from a photograph representing such a circular dish in which the cut-out letters formed the word 'TYPHUS,' and the sharpness of their contour is remarkably strik-

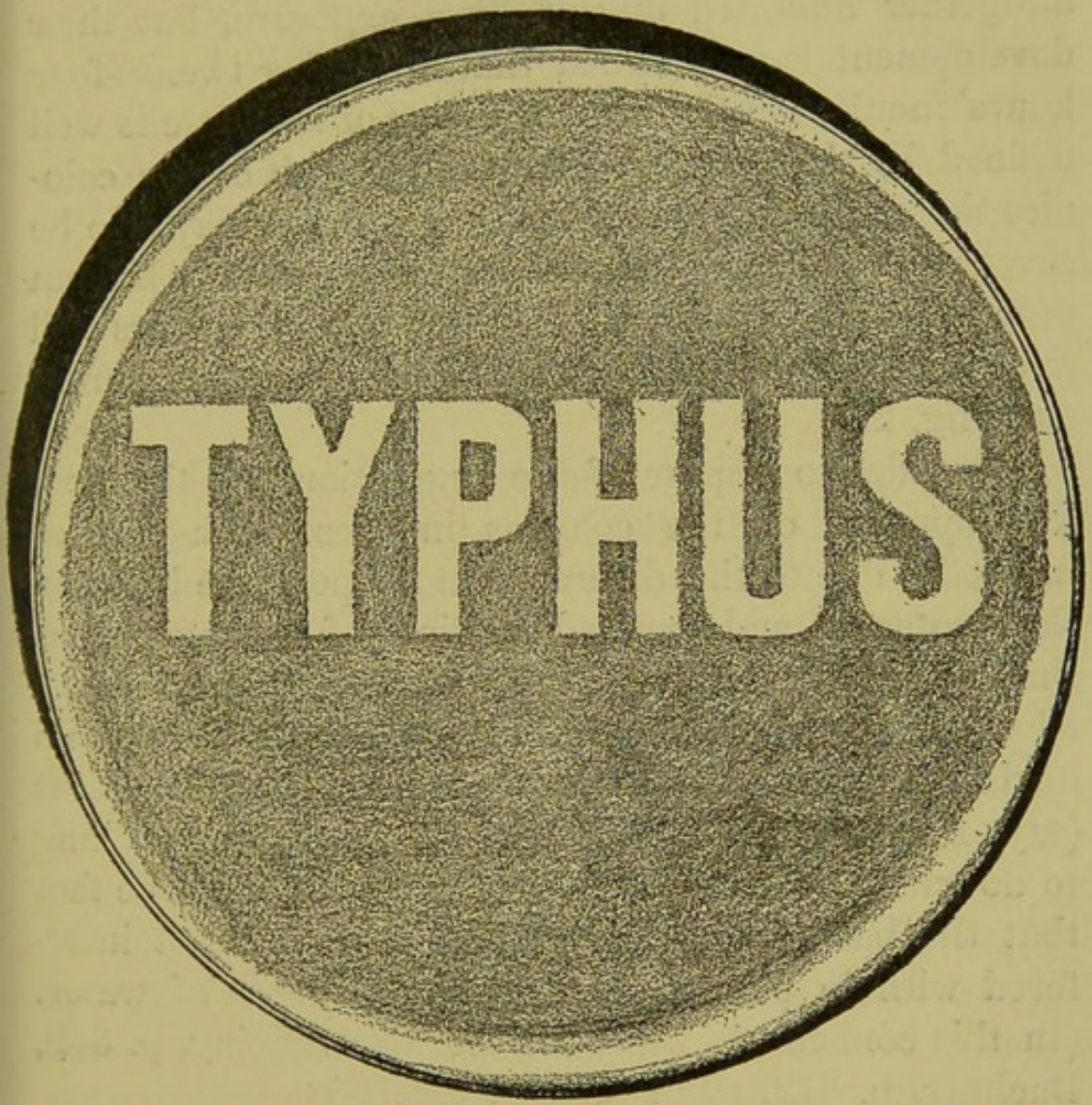


FIG. 22.—TYPHOID BACILLI IN AGAR-AGAR, EXPOSED TO DIRECT SUNSHINE FOR ONE HOUR.
(After Buchner.)

ing. In order to obtain such sharp *photobacteriographs*, as we propose to call them, Buchner points out that the culture material must be thickly sown with the particular bacteria, so that the resulting colonies shall

be as small and closely packed as possible. If the individual centres are able to develop into large colonies, they will naturally extend beyond the margin of the screened part, the sharpness and definition of the letters being thereby impaired. Even ten minutes' direct insolation is sufficient to exhibit the letters of the screen, although the production of colonies is not altogether inhibited by this short exposure, but their development is so retarded that even after twenty-four hours' incubation the protected part of the plate is well defined by the far more luxuriant growth of the colonies there. It is obvious that the process may also be reversed by pasting opaque letters on to the bottom of the transparent dish; and in this case the letters will appear as colonies on the film, instead of as sterile areas as in fig. 22.

In order to dispose of the suggestion that the local development of the colonies in these dish-cultures might be due to the difference in temperature between the protected and exposed portions of the agar-agar, similar dishes were insolated whilst lying in a vessel of water $\frac{1}{2}$ metre in depth, but the results showed no deviation from those previously recorded. This points (says Buchner) not only to temperature having nothing to do with the effect of insolation, but also to the fact that the bactericidal agency of the latter is not interfered with in passing through such a depth of water. (In this connection, however, see also Arloing, p. 342, Buchner, p. 372, and Procacci, p. 374.)

We must now return to the anthrax bacillus, which was the micro-organism selected by Momont for his carefully conducted investigation.[1] The majority of

[1] 'Action de la Dessiccation de l'Air et de la Lumière sur la Bactéridie charbonneuse filamenteuse,' *Annales de l'Institut Pasteur*, vol. vi., 1892, p. 21.

his experiments were made with the sporeless bacilli obtained from the blood of a rabbit dead of anthrax. The effect of desiccation was first tried by introducing single drops of anthrax blood into glass tubes, and subsequently drying them. In some tubes the drop of dried blood was preserved in a vacuum, whilst in others the air was not removed. Such tubes were kept in the dark, some in an incubator at 33° C., and some in a cupboard at from 16° to 22° C. After definite lengths of time had elapsed, nutritive bouillon was added to these tubes, and they were placed in the incubator at 33° C. to see if any growths would make their appearance.

The following results were obtained :—

ANTHRAX BACILLI.—*Desiccation Experiments Conducted in the Dark*

(1) Dried anthrax blood *in contact with air*, preserved in the dark at from 16°–22° C. Maximum duration of life of the bacilli, fifty-seven days. The final bouillon culture obtained in testing the vitality developed only after twenty-four hours. Inoculated into a guinea-pig, it died of anthrax in thirty hours. No attenuation of virulence observed.

In vacuo under similar conditions, maximum duration of life of the bacilli forty-eight days.

(2) Dried anthrax blood *in contact with air*, but kept in the incubator at 33° C. Maximum duration of life of the bacilli, forty-five days. The final bouillon culture obtained developed only after three days. Inoculated into a guinea-pig, it died of anthrax in thirty-seven hours.

In vacuo under similar conditions, maximum duration of life of the bacilli fifty days. Bouillon culture inoculated into a guinea-pig killed it in thirty-six hours of anthrax.

Desiccation Experiments Conducted in Diffused Daylight

(3) Silk threads soaked in anthrax blood and rapidly dried and placed in test-tubes in contact with air, and exposed to diffused light at from 16°–22° C. Maximum duration of life of bacilli, seventy days. The final bouillon culture obtained, when inoculated into a guinea-pig, caused death by anthrax in thirty-six hours. If a silk thread which had been exposed for ten days was, however, introduced directly under the skin of a guinea-pig, no ill effects resulted, but if such a thread was placed in bouillon anthrax growths appeared, and some of this culture inoculated into a guinea-pig invariably induced its death from anthrax. It would appear, suggests Momont, that such dried bacilli when subcutaneously introduced only develop slowly, and before they can do so the migratory cells enclose and destroy them. That the inherent virulence of the bacilli was not destroyed by the above treatment, however, was proved by their subsequent revivification in broth, although such an erroneous conclusion might easily have been arrived at if the negative results obtained by direct inoculation of the threads into guinea-pigs had alone been relied on.

Desiccation Experiments Conducted at High Temperatures

(4) Exposure to a temperature of 55°–58° C. for one hour suffices to destroy all the bacilli present in freshly drawn anthrax blood. If, however, some of this blood was quickly dried, either in test tubes or on cover-glass slips over which it was thinly spread, it was found that the maximum exposure which the bacilli survived was one and a half hour to 92° C., either in the presence or absence of air. The same result was obtained whether the blood was taken from an animal

inoculated with ordinary anthrax or with 'asporogène' anthrax.[1] *Therefore the bacilli of anthrax are capable of resisting far higher temperatures in the dry than in the moist condition.*

SPORELESS ANTHRAX (*Asporogène*).—*Desiccation Experiments with Broth Cultures in Diffused Light and in Darkness respectively*

Instead of anthrax blood, drops of bouillon containing artificially prepared 'asporogène' anthrax were employed.

In dried anthrax bouillon in contact with air, and kept at from 16°–22° C. in diffused daylight, the bacilli preserved their vitality for twenty-one days.

In vacuo under otherwise similar conditions, for seventeen days.

Dried anthrax bouillon cultures of bacilli in contact with air, and preserved in the *dark* at 33° C., preserved their vitality for ten days.

In vacuo under similar conditions, for twelve days.

Thus anthrax bacilli preserve their vitality far longer in dried blood than in dried bouillon. No experiments were made with dried bouillon cultures in the dark at 16° to 22° C.

The same difference was observed when dried bouillon cultures were submitted to high temperatures. Thus thirty minutes' exposure in dried bouillon to 86° C., forty minutes to 80° C., and fifty minutes to 75° C. sufficed to kill the bacilli, whereas in dried blood they only succumbed after ninety minutes' exposure to 92° C.

It is to be regretted that Momont has not recorded

[1] Chamberland and Roux made the interesting discovery that anthrax bacilli were unable to produce spores in bouillon to which a small amount of phenol had been added. Such sporeless bacilli (or *asporogène* anthrax) are quite as virulent as the ordinary anthrax bacilli.

absolutely parallel and comparable experiments with ordinary anthrax bacilli and asporogène bacilli respectively in both blood and bouillon, and that he confines himself to the bare statement that both these varieties of anthrax bacilli exhibit the same degree of resistance.

Desiccation Experiments in Sunlight with Broth Cultures of Asporogène Bacilli

The dried bouillon cultures were prepared as previously described. The maximum duration of life of the bacilli in contact with air = 5 to $5\frac{1}{2}$ hours. *In vacuo* under similar conditions, maximum duration = $6\frac{1}{2}$ hours. The culture employed was only twenty-four hours old, and it was found that the younger the culture the greater was its resistance. The temperature recorded during the period of insolation varied between 25° and 35° C.

Desiccation Experiments in Sunlight with Anthrax Blood

(1) Maximum duration of the bacilli in contact with air = 8 hours. The development of the last culture was delayed for forty-eight hours.

In vacuo under similar conditions, maximum duration=11 hours. The development of the last culture was delayed for twenty-four hours. In both cases the bouillon cultures obtained from the insolated blood killed guinea-pigs in thirty-six hours. In another series of experiments bits of sterilised blotting-paper were soaked in anthrax blood and insolated in test-tubes. This blotting-paper, insolated for from one, two, three, up to fifteen hours, introduced under the skin of guinea-pigs killed them in from thirty-six hours to three days. When insolated for sixteen hours this infected paper no longer killed guinea-pigs, although in bouillon it gave

rise to virulent cultures. When the anthrax blood was thinly spread over a cover-glass, an insolation of six and a half hours was sufficient to destroy its lethal properties, for when fragments of this glass were introduced under the skin of a guinea-pig no symptoms of anthrax made their appearance. Bits of the same glass, however, placed in bouillon gave rise to virulent cultures. Thus, in neither instance were the bacilli actually destroyed, but their pathogenic properties were extinguished, although capable of revivification. The fibres of the paper served apparently to protect the bacilli from the full effects of insolation, the cover-glass exposures losing their virulence far more rapidly.

In these experiments the greater sensitiveness of the bacilli enclosed in bouillon to those present in blood is again shown very distinctly.

Experiments in Sunlight on Asporogène Anthrax in Moist Surroundings

Into each of a series of glass tubes one drop of an asporogène anthrax bouillon culture was introduced; the tubes were then sealed up, remaining full of air. Into other small tubes about $\frac{1}{2}$ c.c. of such bouillon culture was introduced; these tubes being completely filled with the liquid contained no air, and the apertures were sealed in the blowpipe flame. The maximum duration of life of the bacilli in the moist condition insolated in the *presence of air* was two and a half hours; whilst the bacilli preserved in a moist condition, but in the *absence of air*, were still living after insolation for fifty hours.

These results show very strikingly that the agency of light is immensely increased when acting in combination with air. The last cultures obtained were very virulent when inoculated into guinea-pigs.

Momont concludes his most interesting memoir with an account of the behaviour of anthrax spores in sunlight.

ANTHRAX SPORES.—*Action of Light on*

Dried spores insolated in the presence of air for more than 100 hours yielded broth cultures, the development of which was delayed for from one to four days. These cultures were very virulent. Dried spores obtained from the same source and insolated for 100 hours *in vacuo* after being inoculated into bouillon yielded very virulent cultures.

Thus dried spores resist the action of sunlight for a long time, whether in the presence or absence of air. We shall describe later on Momont's experiments on the behaviour of anthrax spores when insolated in water (see p. 371).

These extremely interesting and suggestive investigations were conducted by Momont in M. Roux's laboratory in the Institut Pasteur. It will be remembered that it was Roux who first demonstrated conclusively the changes in the culture medium which may be induced during insolation; to eliminate this difficulty and render the problem less complicated, and ascertain, if possible, the exact effect produced by insolation on the anthrax bacteria themselves, Momont has invariably tested the vitality of the insolated specimens by subsequently introducing them into fresh bouillon preserved from light. If they were found incapable of revivification by this means, then, and only then, was it assumed that they were destroyed. The mere fact that during insolation the bouillon remains clear is not a guarantee that the contained anthrax organisms are killed, as has been assumed by many observers; it may only mean that the culture me-

dium has been so interfered with during insolation that it no longer affords a suitable nourishing material for the micro-organisms therein which may yet be in a living condition. It will be obvious, therefore, that these experiments of Momont's have distinctly advanced our knowledge of this complicated subject in many directions; and the confirmation of the fact suggested by previous observers that the nature of the culture medium in which the organisms are contained is of importance is very strikingly worked out for bouillon and blood respectively.

It will also be noted that Momont does not confirm Arloing's observations that the spores of anthrax are more susceptible to light than the bacillar form, for he finds that, *whilst the dried spores will withstand direct sunlight for over* 100 *hours either in the presence or absence of air, the dried bacillar forms succumb after eight hours' insolation in the presence of air and eleven hours in the absence of air when the bacilli were dried in blood, and five and a half hours' insolation in the presence of air, and six and a half hours in the absence of air, when the bacilli were dried in bouillon. In no case also was any permanent attenuation of the virus obtained by insolation.*

Marshall Ward [1] has more recently exposed the dried spores of anthrax in Petri dishes with alphabetical screens as employed by Buchner (see p. 361), the spores being spread on the glass surface in the absence of all food materials; after insolation a slab of solidified agar-agar was placed on the film of spores and the whole incubated, with the result that only the spores which had been protected by the screen germinated in the agar-agar. On the other hand, in a converse experiment, a slab of agar-agar was first insolated and then laid on the film of spores which had not been exposed

[1] *Proc. Roy. Soc.* vol. liii., 1893, p. 310.

to sunlight; on incubation the spores germinated uniformly throughout the dish. This shows, on the one hand, that the dry spores are acted upon by light in the absence of food materials, and on the other that the particular degree of insolation to which the agar-agar was subjected did not appreciably diminish its nutritive value as regards the germination of anthrax spores. The results are, therefore, confirmatory of those originally obtained by Downes and Blunt with casual mixtures of bacteria.

The Action of Light upon Micro-organisms in Water

So far we have been considering the action of light upon various micro-organisms when present in different culture media, such as broth, gelatine, agar-agar, and potatoes ; but we must now survey what has been done in ascertaining the behaviour of micro-organisms during insolation in various waters. The literature on this branch of the subject is exceedingly meagre. Straus, in the paper already referred to, states that anthrax spores are able to resist the effect of insolation in distilled water over a long period.

Arloing,[1] in carrying out investigations to meet the criticisms of Straus, made the following experiments with anthrax spores in water. Flasks containing sterile distilled water were inoculated with spores ; two were kept in the dark, whilst the remainder were exposed to the action of the sun in February. After varying lengths of time the flasks were withdrawn, and nutritive bouillon added to the water, after which they were placed in the incubator and kept at 35° C. Last of all broth was added to the flasks kept in the dark, and

[1] 'Les Spores du B. Anthracis sont réellement tuées par la Lumière solaire,' *Comptes rendus*, vol. civ., 1887, p. 701.

these were then likewise transferred to the incubator. It was found that in those flasks which had been insolated for 6 and 9 hours respectively, as well as in the control flasks kept in the dark, abundant growths made their appearance. In the flasks insolated during 12 hours the vegetation was more scanty; whilst those flasks which had been insolated during 16, 24, 27, and 30 hours respectively remained entirely free from growths.

Momont, in order to investigate the effect of insolation on anthrax spores in a moist condition, where their vitality would not be interfered with by the alteration in the medium during insolation, introduced such spores into pure water. One such inoculated drop of water was placed in a test-tube and insolated; it was found that 44 hours' exposure to direct sunshine was sufficient to destroy the spores, whilst similar experiments conducted in vacuum tubes revealed the fact that, in the absence of air, anthrax spores in water can withstand insolation for over 110 hours, and retain, moreover, their virulence.

Buchner [1] conducted a number of experiments on the vitality of various organisms in distilled and ordinary tap water during insolation. This investigator states that on all the varieties of bacteria, typhoid bacilli, B. coli communis, B. pyocyaneus, cholera bacilli, and various putrefying bacteria, which he introduced into and examined in water, light exercised a most markedly deleterious effect. The following is the only experiment quoted by this author:—A water into which about 100,000 germs per c.c. of B. coli communis had been introduced at the commencement of the experiment, after one hour's exposure in direct sunlight con-

[1] 'Ueber den Einfluss des Lichtes auf Bakterien,' *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 781.

tained none whatever. In the control vessel exposed under the same conditions, but from which the light was excluded by drawing a covering of black paper over the flask, the contained organisms had undergone a slight increase. Diffused daylight had a less powerful effect than sunlight, but also produced a marked effect after a few hours, and in some cases succeeded in destroying all the organisms present.

In a more recent paper[1] Buchner has endeavoured to ascertain at what depth in water the bactericidal action of light ceases. For this purpose he used the method described on p. 360. Recently infected agar-agar dishes, partly covered with a leaden cross, were lowered to particular depths in the Starnberger Lake, near Munich; the site selected for the experiments was the starting-place of the steamers, and the water was not quite clear. The following table shows the results obtained:—

Action of Sunlight During 4½ *Hours on Bacteria at Different Depths of Water* (Buchner)

(Temp. of Water = 15° R.)

Depth of the Dish in the Water	Particular Organism used	Development of the Colonies	
		In the Shaded Part of the Dish	In the Exposed Part of the Dish
0·1 metre	Cholera bacillus	Very strong . .	None
1·1 „	B. pyocyaneus	„ „ . .	„
1·6 „	Typhoid bacillus	„ „ . .	„
2·6 „	B. pyocyaneus	Decidedly stronger than in exposed part of dish	Fairly strong
3·1 „	Typhoid bacillus	Slightly stronger than in exposed part of dish	Strong

At a depth of 1·6 m. the bactericidal action of the sun's rays is as strong as outside the water, but at 2·6 the action was much less apparent, whilst at 3·1 it was only just perceptible.

[1] 'Ueber den Einfluss des Lichtes auf Bakterien und über die Selbstreinigung der Flüsse,' *Archiv für Hygiene*, 1893.

It is to be regretted that Buchner did not employ one variety of organism only at the various depths, as by not doing so he has introduced another factor, it being quite possible that each variety of organism is endowed with individual powers of resistance to insolation. The experiments show, however, very clearly that the sun's rays only exert a bactericidal action in the upper layers of water, and we know that bacteria are present in large numbers at depths very considerably below that within the reach of insolation, so that sunshine must exert only a superficial effect in the bacterial purification of water.

Buchner includes in his paper some investigations carried out towards the end of September 1892 by Minck and Neumayer on the bacterial condition of the river Isar during the day and night respectively. These experiments were made to find out if the number of microbes in the river water during the night, and therefore in the absence of the deleterious influence of light, was greater than in the daytime. The spot selected was 10 km. above Munich, and the gelatine plates were prepared immediately after the collection of the samples. The following table gives the results of their examinations :—

Bacterial Condition of the River Isar in the Day and Night Respectively (Minck and Neumayer)

Time of Taking Sample	Number of Organisms on 1 c.c. of Water
$6\frac{1}{4}$ evening	160
$8\frac{3}{4}$ „	5
11 „	8
12 „	107
$1\frac{3}{4}$ morning	380
3 „	460
4 „	520
5 „	510
$6\frac{1}{4}$ „	250

It is obvious that an investigation of this kind must

be undertaken with extreme precaution, and that in the interpretation of the results it must not be forgotten that numerous other factors besides the presence or absence of light contribute to bring about numerical differences in the bacteria present. It is therefore desirable that further confirmation of the above results should be forthcoming before any decided inference can be drawn. In this connection it may be pointed out that we have repeatedly found about twenty times as many microbes in the waters of the rivers Thames and Lea during the winter as in the summer months (see table, p. 123). Here, again, we must recognise that there are other agencies besides the greater duration and potency of the sun's rays in the summer also tending to reduce the number of bacteria present at this season of the year, although sunshine may undoubtedly have contributed to bring about the observed contrast.

Procacci [1] has still more recently investigated the depth to which the lethal action of the sun's rays extends in water. For this purpose ordinary drain-water was employed. Cylindrical glass vessels about 60 cm. high and 25 cm. wide were filled with drain-water; from some of these the light was excluded, whilst in others it was allowed free access. The temperature in the exposed vessels never exceeded that of the darkened by more than from 2° to 4° C., and in neither case did it ever rise beyond from 40° to 42° C. In every instance a marked diminution in the bacterial contents was observed in the insolated vessels, whilst at the same time a more or less decided increase took place in the darkened ones, the duration of the experiments varying from $1\frac{1}{2}$ to 9 hours. In order to ascertain to what depth the antiseptic action of sunlight extended, and what part was played by the

[1] 'Influenza della luce solare sulle acque di rifiuto,' *Annali dell' Instituto d'Igiene Sperimentale di Roma*, vol. iii., 1893, p. 437.

perpendicular and oblique rays respectively, Procacci investigated their action separately as well as together. When the vessels were exposed to the perpendicular as well as the oblique rays of the sun, the bactericidal power of the light was unimpaired at the bottom of the vessel, at a depth of half a metre; but when the perpendicular rays only were admitted, this power ceased, the bacteria near the surface only being destroyed, whilst in the lower layers they remained nearly as numerous as in the cylinder which was not insolated at all. The following table shows the actual results obtained :—

Effect on Bacteria of Sunshine Passing Vertically through 60 *cm. of Drain-water* (Procacci)

June, 1893, 12 noon–3 P.M.	Before Exposure	Bacteria in 1 c.c. of Water	
		After 3 hours' Sunshine	Darkness
Surface . .	2,100	9	3,103
Centre . .	2,103	10	3,021
Bottom . .	2,140	2,115	3,463

That the oblique rays rendered important service in the destruction of the bacteria at the bottom was further shown by a special examination of portions of the water in the immediate vicinity of the sides of the cylinder, for on freely admitting these rays and excluding the perpendicular, it was found that the smallest number of bacteria was present in those parts of the water which were nearest to the glass.

A systematic series of experiments on the bactericidal action of light on organisms in water was made by one of us[1] in connection with the behaviour of sporiferous anthrax introduced into various waters. These results are of especial interest, as not only was the *vitality*

[1] 'Experiments on the Vitality and Virulence of Sporiferous Anthrax in Potable Waters,' Percy Frankland, *Proc. Roy. Soc.*, vol. liii., 1893, p. 204.

of the anthrax cultures tested in the various waters after insolation, but their *virulence* was directly determined by inoculation into white mice.

The water used in these experiments was obtained from the river Thames and was employed unsterilised, after filtration through Swedish paper, after filtration through porcelain (Chamberland), and after steam sterilisation. The flasks containing these several descriptions of water were inoculated with anthrax spores and kept in the incubator (18°–20° C.) or refrigerator (6°–10°C.). In the description of the results obtained, those flasks against which an 'I' is placed were put in the incubator, whilst those marked 'R' were preserved in the refrigerator. The numerals appended to the letters indicate the particular flask examined in each of the several series.

Vitality and Virulence of Sporiferous Anthrax in Thames Water Exposed to Diffused Light (Percy Frankland)

All the flasks employed in these investigations had been in the refrigerator or incubator from the day of infection with anthrax (March 18, 1892) until March 25, 1892, from when they remained in a dark room until April 9, 1892, after which they were exposed to the diffused daylight in a room with a southern aspect.

The anthrax in the previously sterilised (porcelain and steam) Thames water survived this exposure of upwards of two months to diffused daylight, nor did the number of colonies obtained on plate cultivation differ materially from that obtained from the corresponding flasks preserved throughout in the dark. Indeed, by direct experiments on animals (see pages 377–379) it was shown that the anthrax remained both alive and

virulent in these waters, after upwards of six months' exposure to diffused daylight.

On the other hand, the degeneration of the anthrax in the unsterilised Thames water was observed to be distinctly more rapid in these flasks exposed to daylight than in those preserved in the dark. Thus, in the case of the unfiltered Thames water (daylight) the special method (see page 283) of examination revealed no anthrax from May 17, 1892, whilst in the same water, kept both in the incubator and refrigerator, anthrax was discovered by the same method on July 9, 1892.

The following experiments were made to test the virulence of the flasks which had been thus exposed to diffused daylight:—

Animal Experiment.—On October 8, 1892, 1 c.c. of water from the flask '1 I, Thames water, unfiltered, infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse did not succumb, but was alive thirty-two days after the operation.

This result was to be anticipated, seeing that the corresponding flasks 3 I and 3 R, which had not been exposed to daylight, also failed to kill mice.

Animal Experiment.—On October 7, 1892, 1 c c. of water from the flask '5 I, Thames water, paper-filtered, infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse did not die, but was still alive thirty-three days after the operation.

A corresponding flask, 1 R, which had not been exposed to daylight did kill a mouse, so that the virulence had in this case been reduced by the exposure.

Animal Experiment.—On October 7, 1892, 1 c.c. of water from the flask '5 I, Thames water, porcelain-filtered, infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse died within 6 days 20½ hours. The body exhibited extensive œdema ; the spleen was only slightly enlarged, but was found to contain anthrax bacilli both microscopically and by cultivation in gelatine.

Animal Experiment.—On October 15, 1892, 1 c.c. of water from the flask '5 I, Thames water, steam-sterilised, infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse died within 4 days 17 hours; the body exhibited much œdema, and the spleen was not very large; anthrax bacilli were detected in the latter both with the microscope and by cultivation in gelatine.

The contrast exhibited by the sterilised and unsterilised Thames water is thus most striking in the case of these flasks exposed to daylight, for both the unfiltered and paper-filtered waters failed to kill, whilst the porcelain-filtered and the steam-sterilised waters were fatal to the mice into which they were injected. The lethal effect of both the latter, and especially of the porcelain-filtered water, accompanied by the non-typical symptom of only slight enlargement of the spleen, points to an attenuation of the virus.

These results did not lead me to conclude, however, that the anthrax virus was necessarily quite extinct in these two unsterilised waters (viz., the unfiltered and paper-filtered Thames water), and I resorted, therefore, to the method before employed (see p. 283) of revivifying it by the addition of 5 c.c. of sterile broth to each of the two flasks in question.

The flasks so treated were placed in an incubator at 37° C., and the following further experiments made with them :—

Animal Experiment.—On October 22, 1892, 0·5 c.c. of the water (to which broth had been added on October 15, 1892) in the flask '1 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse died within 2 days 18 hours. The body exhibited extensive œdema and the spleen was much enlarged; the latter was found full of anthrax bacilli, the presence of which was confirmed by cultivation in gelatine.

Animal Experiment.—On October 18, 1892, 0·5 c.c. of the water (to which broth had been added on October 15, 1892) in the flask '5 I, Thames water, paper-filtered, and infected with anthrax March 18, 1892, exposed to daylight since April 9, 1892,' was subcutaneously injected into a white mouse. The mouse died within 1 day 19 hours. Only few bacilli were found in the spleen, but more in the kidney; their presence was confirmed by gelatine cultivations from both organs.

These experiments show, then, *that in the flasks in question (unsterilised Thames water exposed for upwards of six months to diffused daylight), although the number of anthrax germs had been so far reduced that* 1 *c.c. would not kill mice, yet after nourishment with broth they were so revivified as to be fatal to these animals when injected in the same or even a smaller quantity.*

Vitality and Virulence of Sporiferous Anthrax in Thames Water Exposed to Direct Sunshine (Percy Frankland)

The flasks infected with sporiferous anthrax bacilli on March 18 were taken from the incubator and refrigerator respectively on March 25, 1892; they remained in a dark room from that day to April 9, 1892, and from then onwards they were exposed to as much sunshine as could be conveniently obtained, and which was approximately estimated in hours, although it is obviously very difficult to make any exact determination of the latter. The temperature of the water so exposed never exceeded 32° C.

The results, which are very striking, are easily followed, thus:—

Unfiltered Thames water, anthrax was still alive on May 2, 1892, after 56 hours' sunshine, but extinct on May 12, 1892, after about 84 hours' insolation.

Paper-filtered Thames water, anthrax was almost extinct on May 15, 1892, after about 92 hours' insolation, and quite extinct on June 17, 1892, after about 151 hours' sunshine.

Thames water filtered through porcelain, anthrax was still alive on May 2, 1892, after about 56 hours of sun, but extinct on May 12, 1892, after about 84 hours' insolation.

Thames water sterilised with steam, anthrax was still alive on May 2, 1892, after about 56 hours', but dead on May 12, 1892, after about 84 hours' sunshine.

In consequence of the sunshine having destroyed the greater number of those water bacteria causing liquefaction of the gelatine, it was possible to incubate the plates for a long period of time, and thus in most instances to dispense with the special method of examination by preliminary heating.

The above results have only reference to the presence or absence of anthrax as revealed by cultivation, but experiments were also made on the virulence of these waters which had been exposed to direct insolation. Thus :—

Animal Experiment.—On November 2, 1892, 1 c.c. of the water from the flask '4 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Animal Experiment.—On November 2, 1892, 1 c.c. of the water from flask '4 I, Thames water, steam-sterilised, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Animal Experiment.—On November 2, 1892, 1 c.c. of the water from flask '4 I, Thames water, porcelain-filtered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Thus, in all three cases, the water was non-virulent when injected to the amount of 1 c.c. It was, however, obviously not to be necessarily concluded that the anthrax had become absolutely extinct in these waters, and in order to put this point to the test the flasks in question were each treated with 5 c.c. of sterile broth and incubated at 37° C., after which the following further experiments were made :—

Animal Experiment.—On November 9, 1892, 0·5 c.c. of the water (to which broth was added on November 7, 1892) from flask '4 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Animal Experiment.—On November 9, 1892, 0·6 c.c. of the water (to which broth was added on November 7, 1892) from flask '4 I, Thames water, steam-sterilised, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Animal Experiment.—On November 9, 1892, 0·6 c.c. of the water (to which broth was added on November 7, 1892) from flask '4 I, Thames water, porcelain-filtered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,' was subcutaneously injected into a white mouse. The mouse remained alive.

Thus in these waters exposed to direct sunshine for 151 *hours the anthrax germs were completely destroyed, and could not be revived by the addition of broth.*

The destruction of anthrax spores by direct sunshine is a subject which, as we have seen, has received the attention of a number of observers. Thus, Arloing ('Compt. rend.,' vol. c., 1885, p. 378, and vol. ci. p. 511) found that they were destroyed in two hours, whilst in subsequent experiments in which the spores were placed in broth maintained at a temperature of 4°–11° C. by means of ice five hours' insolation effected their destruction. Roux ('Ann. de l'Inst. Past.,' 1887, p. 445) again insolated the spores when dispersed in the aqueous humour of the ox-eye, and found them destroyed in from twenty-nine to fifty-four hours, whilst Pansini ('Rivista d' Igiene,' 1889) observed their destruction on potatoes in from four to five hours, in gelatine in from six to seven hours, and in broth in from thirty minutes to two hours. Ward ('Proc. Roy. Soc.,' vol. lii. p. 393) has also more recently demonstrated their destruction in from two to six hours when insolated in agar-agar, exposed in circular glass dishes.

In these experiments Buchner's method was employed. In all these experiments it will be seen that nutrient culture media were employed for the insolation, and that the spores were destroyed in a much briefer period of time than in my experiments, in which they were insolated in Thames water. This same phenomenon of the *spores of anthrax being more resistant to the action of sunshine in water than in ordinary culture materials* has also been observed by Straus ('Société de Biologie,' 1886, p. 473) and by Momont ('Ann. de l'Inst. Past.,' 1892, p. 21), who both, however, appear to have made use of distilled water only.

This greatly increased resistance to insolation which is exhibited by bacteria when suspended in water instead of in culture media, is of great importance from a practical point of view. In the first place we would, however, point out how fallacious must be any comparison between the length of insolation withstood by even one and the same micro-organism in the hands of different observers, as so much depends upon their previous history and treatment. Thus it was found by one of us that the spores of anthrax produced at from 18° to 20° C. are far more resistant than anthrax spores obtained at from 35° to 38° C. In all comparative experiments, therefore, the organisms should be taken from one and the same cultivation.

In endeavouring to ascertain the cause of the greater susceptibility of bacteria to light when exposed in culture media, we are proceeding by way of synthesis, making various additions to distilled water, and then determining how such additions affect the influence of insolation.[1] In this manner we have already made some preliminary experiments with common salt and

[1] *British Association Reports*, 1893; also *Centralblatt für Bakteriologie*, vol. xv., 1894, p. 111.

sodium sulphate. Some of the results already obtained are given in the following table :—

Action of Sunshine on Anthrax Spores suspended in Water
(Percy Frankland)

Spores produced at 18°–20° C.				Spores produced at 38° C.			
3 hours' sunshine, 240 per c.c.		Darkness, 490 per c.c.		3 hours' sunshine, 4 per c.c.		Darkness, 476 per c.c.	
NaCl	Na_2So_4	NaCl	Na_2So_4	NaCl	Na_2So_4	NaCl	Na_2S
1 % 117	239	450	474	1 % 0	0	314	390
3 % 81	218	384	426	3 % 1·5	1	132	343
10 % 46	187	150	622	10 % 0	0	115	220

Thus the bactericidal action of light is very considerably greater in water containing common salt than in distilled water; whilst, on the other hand, the addition of sodium sulphate in the same proportions has little or no influence in this respect. An addition of 10 per cent. of salt appears to exercise even some bactericidal effect in the dark. The action of this material in enhancing the bactericidal action of light is still more conspicuously brought out in the following experiments :—

Action of Sunshine on Anthrax Spores suspended in Water
(Percy Frankland)

Spores produced at 18°–20° C.

	Sunshine. Number per c.c.				Darkness. Number per c.c.			
	No additions	1 % NaCl	3 % NaCl	10 % NaCl	No additions	1 % NaCl	3 % NaCl	10 % NaCl
4 hours	16,000	14,000	8,000	5,000	13,000	13,000	9,000	12,000
11 hours	12,000	8,000	3,000	485	15,000	13,000	16,000	14,000
21 hours	378	39	49	0	18,000	15,000	14,000	9,000

The Action of Light on the Virulence of Pathogenic Bacteria

We mentioned on p. 340 that Arloing found incidentally that anthrax cultures, after insolation for about 30 hours, were not only less virulent when inoculated into animals, but that they acted under such circumstances as a sort of vaccine; guinea-pigs not only surviving inoculation with them, but acquiring also a more or less pronounced degree of immunity towards the action of virulent anthrax cultures.

Very few investigations have been made in this interesting and important branch of the subject; indeed, since the publication of the above observation, made by Arloing in 1885, no experiments in this direction appear to have been undertaken until the year 1891, when Kitasato[1] examined the degree of virulence possessed by the filtrate of tetanus-broth cultures when exposed to the light and dark respectively. This investigator found that in diffused light these tetanus filtrates (*i.e.*, the broth from which the tetanus bacilli had been removed by filtration through porous porcelain) by degrees lost their toxic properties; it was, however, a slow and gradual process, for even after standing from nine to ten weeks in diffused light they were still feebly toxic. On the other hand, similar tetanus filtrates preserved in the dark were still, after being kept for 300 days, as virulent as when they were originally prepared. In direct sunshine, at a temperature of from 35° to 43° C., the toxic properties were entirely destroyed in from 15 to 18 hours.

This question has been still more recently in-

[1] 'Experimentelle Untersuchungen über das Tetanusgift,' *Zeitschrift für Hygiene*, vol. x., 1891, p. 285.

vestigated by Fermi and Pernosi,[1] who also used tetanus filtrates obtained both from gelatine and broth cultures. In these experiments the temperature during insolation was from 38° to 41° C., and the toxic properties were found to be destroyed by from 8 to 10 hours' exposure to sunshine. When, however, the temperature during insolation was not allowed to rise beyond 37° C. (which was secured by keeping the tubes containing the filtrate immersed in water), it required 15 hours' sunshine to remove the pathogenic properties. On drying, however, the filtrate in a desiccator and exposing it in Petri dishes to sunshine, 100 hours' insolation did not destroy the toxic properties, similar results being obtained when chloroform, ether, benzene, and amyl alcohol were respectively added to the dried filtrate before insolation.

Palermo [2] has also published some exceedingly interesting investigations on the action of sunshine on the *virulence* of the cholera bacillus when suspended in broth and sterilised distilled water respectively. The initial virulence of all the infected liquids employed, both broth and water, was in each case ascertained before insolation by inoculation into guinea-pigs, and the virulence of the insolated and non-insolated liquids was afterwards similarly determined and compared with that exhibited by the liquids which had been kept under the ordinary conditions, but had not been placed in the sunshine either in protected or unprotected vessels. The following table shows the action of sunshine on the pathogenic properties of the cholera bacillus :—

[1] 'Sul veleno del tetano,' *Annali dell' Istituto d' Igiene Sperimentale di Roma*, vol. iv., 1894, p. 8.

[2] 'Azione della Luce solare sulla virulenza del bacillo del Colera,' *Annali dell' Istituto d' Igiene Sperimentale di Roma*, vol. iii., 1893, p. 463.

Effect of Sunshine on the Virulence of the Cholera Bacillus suspended in Broth (Palermo)

Exposure to Sunshine	Effect on Guinea-pigs
0	Animal died in 18 hours.
10 min.–2 hours . .	„ „ „
3 hours	One animal died in 18 hours, but another, also inoculated with the same liquid, died after 5 days.
$3\frac{1}{2}$–$4\frac{1}{2}$ hours . . .	Remained alive.

The interesting discovery was further made that those animals which had survived inoculation with the insolated cholera cultures had thereby become protected from the pathogenic action of virulent cholera bacilli, for when 8 days later they were inoculated with the latter, instead of dying, as is usual, in about 18 hours, they remained alive. It will be remembered that Arloing claimed to have reduced virulent anthrax cultures to the condition of vaccine by insolation, but, so far as we are aware, Palermo is the only other investigator who has been able to render animals artificially immune to a disease by inoculation with the insolated bacteria. When instead of broth sterilised distilled water was used, the pathogenic properties of the cholera bacillus were more rapidly destroyed (in from 2 to 3 hours) under insolation.

Palermo also determined the effect of sunshine during the various periods of exposure on the *number* of cholera bacilli present, as indicated by gelatine cultures, but in no instance could any difference in the numbers present be detected. On prolonging the exposure to from 6 to 7 hours however, although no effect was produced on the numbers present in the insolated broth cultures, yet the physiological character of the bacilli had undergone considerable modification, for exposure to sunshine was found to have deprived them

of all power of motility, whilst the characteristic activity was still apparent in those drop-cultures prepared from the tubes which had remained in darkness.

From the experimental data which we have recorded in the preceding pages, it will be seen that the work inaugurated by Downes and Blunt in 1877 has been followed up by a large number of investigators, and that the information concerning this action of light on micro-organisms is in some respects already remarkably complete. In examining the work which has been done in connection with this subject by so many investigators, it is impossible not to be struck with admiration at the remarkable intuition, prescience, and experimental acumen exhibited by the pioneers in this department, and we are of opinion that the work of Downes and Blunt should be regarded as one of the most complete and successful bacteriological investigations made before the introduction of the modern methods of studying micro-organisms. In their experiments they so completely realised the various factors entering into this question that there is hardly a single new point of importance which has been brought out by more recent investigations, and the latter, whilst further elucidating many details, have in the main confirmed all the results of the original investigators.

If we endeavour to summarise the results which have hitherto been obtained, we shall find that, in spite of some apparent contradictions and discrepancies, the present position of this subject is on the whole very definite.

Thus—

1. There is no question that light, and more especially sunlight, has a deleterious effect on bacteria in their vegetative, and to a less extent in their spore, forms. This has been established not only by Downes

and Blunt's original experiments on casual mixtures of micro-organisms, but also by numerous experiments in which pure cultivations of the most diverse microbes have been employed.

2. This deleterious effect can be produced by light irrespectively of the rise in temperature which must accompany direct insolation unless special precautions be taken. It is, moreover, the most highly refrangible rays of the spectrum that are the most injurious to bacterial life, the ultra-violet being the most, and the infra-red the least, powerful in this respect, a circumstance which clearly indicates that the phenomenon is due to chemical action.

3. It was already shown by Downes and Blunt, and has been abundantly confirmed by Roux and Momont, that the action of light is greatly increased by the simultaneous presence of air and moisture; indeed, so pronounced is the influence of oxygen in this action that there can be no doubt that the effect is due to a process of oxidation, possibly brought about through the agency of ozone or peroxide of hydrogen, or both. This view is supported by some recent experiments of Richardson ('Proc. Chem. Soc.,' 1893, p. 121), in which it is shown that peroxide of hydrogen is formed in urine during insolation, and that the sterilising action of light can be counteracted by the addition of substances, *e.g.* peroxide of manganese, which destroy hydrogen peroxide.

The formation of peroxide of hydrogen during insolation naturally suggests the question whether the whole bactericidal effect of light is due to this material, or whether it only partially accounts for the phenomenon. Richardson has shown that the formation of peroxide of hydrogen is due to the presence of some ingredient or ingredients in the urine, and that it is not

formed by the insolation of water, or even of a solution of urea. If, then, the bacteria are suspended in water during insolation, there can be no generation of peroxide of hydrogen in the liquid. Now, as I have already pointed out in connection with my own experiments ('Proceedings Royal Society,' 1893), a number of investigators are agreed that bacteria are much more resistant to insolation when suspended in water than when suspended in culture materials. It is, however, equally certain that they are actually destroyed, and sometimes even with great rapidity, when suspended in water. Now this at first sight would appear to demonstrate that the bactericidal effect of light, although accelerated by the generation of peroxide of hydrogen, may also take place without it. But in the experiments which have hitherto been made on the action of light on micro-organisms, those conditions have not been secured which entirely preclude the generation of peroxide of hydrogen within the cells of imperfectly dried bacteria and their spores, and it is highly probable that this generation does take place, and it is surely still more easy to believe in the production of this material within the cells suspended in water to which air has access. The question obviously raises another and far more general question, which has long been before the chemical world, viz., as to how far oxidation can take place at all in the entire absence of water; and the evidence on this larger question goes entirely to show that all apparently direct low-temperature oxidations require the presence of moisture. And, inasmuch as the bactericidal action of light is unquestionably a case of low-temperature oxidation, there is the strongest presumptive evidence, as well as weighty experimental evidence, that moisture, which practically means the possibility of the presence of peroxide of

hydrogen or of some similar material, is essential for its manifestation.

4. A complicating factor in the study of the effect of light on bacteria is the action exercised by the light on the medium with which the bacteria are surrounded; but on the one hand it has been distinctly proved that insolation is capable of destroying bacteria when exposed in the absence of any medium (Downes and Blunt, Marshall Ward), whilst on the other hand it has been shown that insolation may so affect some, if not all, culture media as to render them more or less unfit for the cultivation of some micro-organisms. This modification of the culture medium has only been found to take place when the insolation was performed in the presence of air (Roux). It is true that numerous investigators have exposed culture media to insolation without observing any such diminution in their nutritive value, but negative results of this kind do not disprove the positive ones, but only show that the conditions were in some way or other different in the two cases.

5. As regards the precise duration of the exposure to sunlight which is necessary to cause the destruction of any particular bacterial form, the most divergent results have been obtained, not only by different workers, but even by one and the same investigator. That this should be the case is not surprising, for not only is the intensity of the sun's light subject to enormous variations, but also the inherent vitality of one and the same organism differs greatly according to its source and previous history. Thus, it has been shown by one of us that anthrax spores obtained at 38° C. are much more susceptible to the action of light than those which have been obtained from the same original source at 18° to 20° C.

6. There is no evidence that the virulence of anthrax undergoes any permanent attenuation through exposure to light, for although the bacilli or spores which have been insolated, short of being killed, may be incapable of producing a lethal effect on animals, the cultures which can be obtained from them are found to be fully virulent (Roux and Momont).

7. As regards the effect of light on bacteria present in water, distilled or potable, the experiments hitherto made have been principally limited to anthrax spores. Although the exact time that such spores will endure insolation in water varies greatly according to their previous history, the balance of evidence tends to show that they are less rapidly destroyed than when exposed in culture media or in an insolated condition. (The greater power of resisting sunshine in water was indeed already shown for mixtures of bacteria by Downes and Blunt.) Their endurance is particularly long-continued when they are insolated in distilled water in the absence of air, resistance to upwards of 110 hours' exposure having been observed by Momont. It has moreover been shown by one of us that the addition of a halogen salt, in the shape of sodium chloride, to the distilled water materially increases the injurious effect of the sunlight, whilst the addition of an oxy-salt, in the form of sodium sulphate, is practically without influence in this respect.

Of great importance in connection with the deportment of bacteria under insolation in water is the depth to which the sun's rays can take effect. On this point the evidence is very contradictory. Thus, whilst Arloing found that a stratum of water two centimetres in thickness was almost an efficient protective screen for anthrax against the sun's rays, Buchner found that the latter penetrated to a depth of half a metre without having

apparently lost any of their bactericidal effect on typhoid, cholera, and some other bacilli on which he experimented. In some of our own experiments we have found that the sun's rays after passage through a few inches of water were distinctly feebler in their action on anthrax spores than when this impediment was not interposed. Procacci has also shown that the solar rays are largely, if not entirely, deprived of their bactericidal effect by passing through a stratum of water sixty centimetres in depth.

In its special connection with the bacteriology of water we must, therefore, recognise in sunshine, and to a slight extent also in diffused daylight, a powerful bactericidal agency, but one the importance of which there has been a considerable tendency to magnify and exaggerate. On the one hand the experimental evidence shows conclusively that pathogenic bacteria, at any rate anthrax spores, can resist insolation prolonged over many hours and even under the most favourable circumstances, whilst on the other hand it is sufficiently obvious that in a climate like our own a great deal of the surface-water is never exposed to adequate insolation at all, even in the case of shallow streams in which under more favourable climatic conditions this bactericidal agency might be highly effective. Thus, whilst every opportunity should be afforded for insolation in the construction of waterworks, undue reliance must not be placed on this any more than on any other particular bactericidal agency.

APPENDIX

In order to render some assistance in the identification of particular micro-organisms, we have appended the following lists

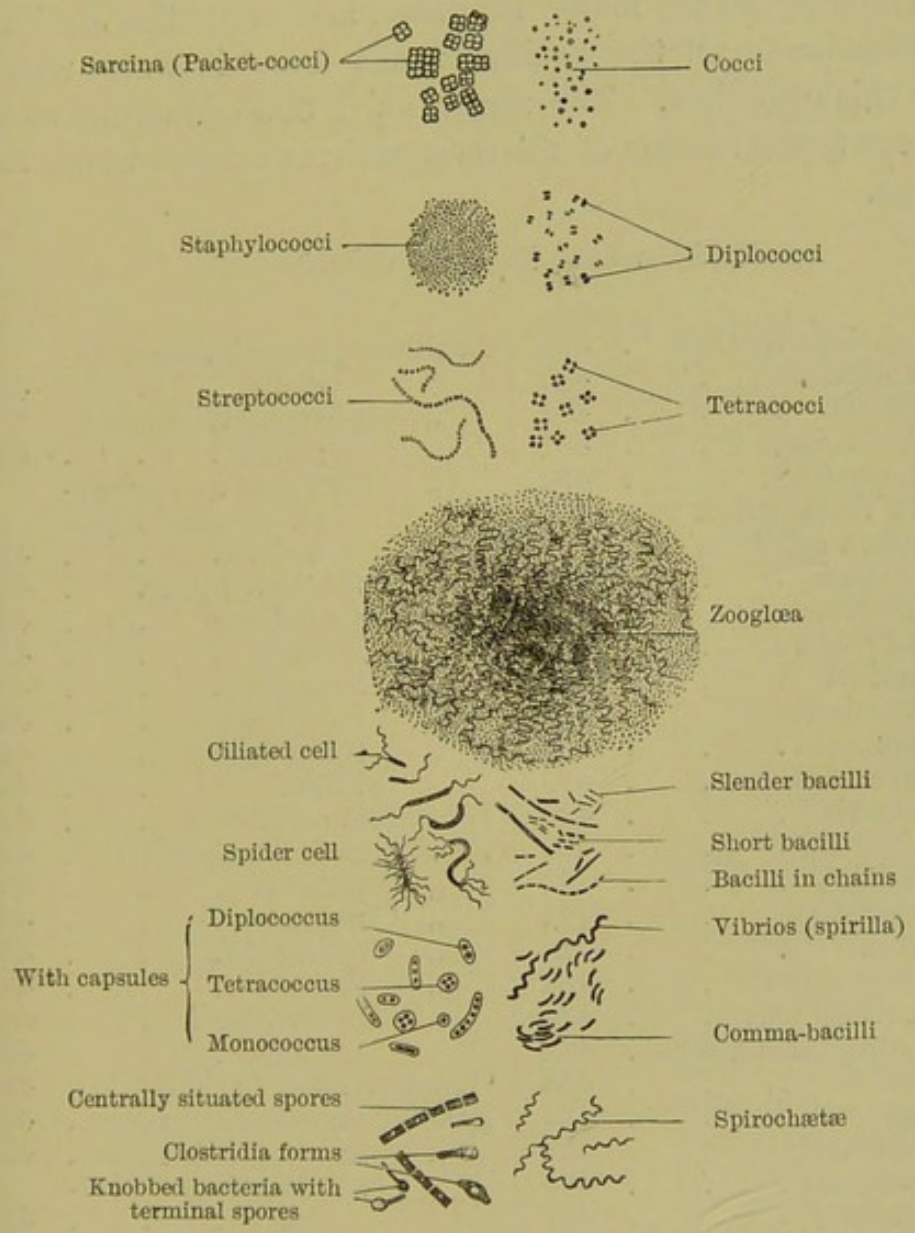


Fig. 23.—Forms of Bacteria. Magnified about 700 times. (After Baumgarten.)

of bacteria, in which, in the first place, will be found bacilli and micrococci separately grouped. These groups have been again divided up into those bacilli which liquefy and do not liquefy

gelatine, and those micrococci which liquefy and do not liquefy gelatine. A list of the bacilli and micrococci respectively which have been found to be pathogenic to man or animals has also been made. The names of such organisms will be found printed in red letters in the text. We have gathered together in sequence as far as possible those organisms which resemble one another in respect of particular pigment produced, whilst we have placed together with the spirillum choleræ asiaticæ all the spirillar forms found in water, and with the bacillus typhi abdominalis all the bacillar forms found in water which resemble this organism. The figure on p. 395 represents some of the more typical forms of micro-organisms as seen under the microscope.

Bacilli which LIQUEFY *Gelatine*

	PAGE
Spirillum choleræ asiaticæ	399
Vibrio Berolinensis	400
Vibrio aquatilis	401
Bacillus choleroides, *a*	401
„ choleroides, *β*	402
'Eine neue Vibrionenart'	402
Pseudo-cholera spirillum	403
Spirillum rugula	407
„ marinum	407
Bacillus anthracis	416
„ subtilis	417
„ vermicularis	418
„ ramosus	419
„ mycoides	419
Proteus vulgaris	420
„ mirabilis	420
„ Zenkeri	421
„ fluorescens	421
Bacillus tetani	423
„ pyocyaneus	424
„ murisepticus	428
„ hydrophilus fuscus	431
„ cloacæ	433
„ superficialis	434
„ reticularis	435
„ circulans	436
„ hyalinus	437
„ delicatulus	438
„ rubidus	440
„ prodigiosus	440
'Der rothe bacillus'	441
Bacillus ruber	442
„ aquatilis (Percy Frankland)	450
Bacillus phosphorescens indicus	451
„ phosphorescens indigenus	452
„ argenteo-phosphorescens liquefaciens	453
„ thalassophilus	468
„ granulosus	454
„ litoralis	455
„ limosus	456
„ halophilus	457
„ tremelloides	447
„ Zopfii	457
Bacterium sulfureum	458
Bacillus radiatus aquatilis	459
„ plicatus	459
„ nubilus	460
„ ochraceus	445
„ liodermos	460
„ liquefaciens	461
„ liquidus	461
'Lemon-yellow bacillus'	445
Bacillus mesentericus ruber	462
„ mesentericus fuscus	463
„ mesentericus vulgatus	463
„ inunctus	464
„ stoloniferus	464
„ incanus	465
„ iridescens	465
„ guttatus	466
„ gen. nov.	467
„ graveolens	467

Bacilli which LIQUEFY *Gelatine*—cont.

	PAGE
Bacillus helvolus	444
„ gasoformans	468
'Yellow bacillus' (Lustig)	446
Bacillus glaucus	469
„ fluorescens liquefaciens	427
„ termo	428
„ fulvus	470
„ filiformis	470
„ diffusus	471
„ devorans	471
„ ianthinus	472
„ membranaceus amethystinus	474
„ cæruleus	474
Bacillus dentriticus	475
„ cuticularis	476
„ 'C' (Foutin)	476
„ carnicolor	477
„ butyricus	478
„ albus putidus	479
'White bacillus' (Maschek)	480
Bacillus crassus aromaticus	480
„ aquatilis graveolens	481
„ arborescens	482
„ aërophilus	482
'Rhine-water bacillus' (Burri)	483
Bacillus megaterium	418
„ putrificus coli	486
„ punctatus	486

Bacilli which DO NOT LIQUEFY *Gelatine*

	PAGE
Vibrio saprophiles, *a*	404
„ saprophiles, *γ*	404
Bacterium luteum	444
'Golden-yellow water bacillus'	444
Vibrio aureus	405
„ flavus	405
„ flavescens	406
Spirillum rubrum	406
„ concentricum	408
Bacillus typhi abdominalis	410
„ coli communis	411
Bacterium tholœideum	412
„ lactis aërogenes	412
Bacillus aquatilis sulcatus, I.	413
„ „ „ II.	413
„ „ „ III.	414
„ „ „ IV.	414
„ „ „ V.	415
Bacillus tuberculosis	422
„ cuniculicida	429
„ brevis	429
„ capsulatus	430
„ saprogenes II.	432
„ ubiquitus	434
„ rubescens	438
„ erythrosporus	439
„ rubefaciens	441
„ cuticularis albus	442
'Weisser bacillus' (Tataroff)	443
Bacillus albus	443
„ aureus	448
„ fluorescens aureus	449
Bacillus aurantiacus	449
'Orange-red water bacillus'	445
Bacillus aquatilis fluorescens	426
„ aquatilis	450
„ amylozyme	469
„ fluorescens non liquefaciens	425
„ fluorescens tenuis	424
„ „ longus	425
„ viridis pallescens	426
„ phosphorescens gelidus	451
„ flavescens	448
„ Berolinensis indicus	473
„ 'D' (Foutin)	476
„ acidi lactici	477
„ lactis cyanogenus	478
„ „ viscosus	479
„ brunneus	432
„ constrictus	484
„ flavocoriaceus	446
„ fuscus	431
„ fuscus limbatus	432
„ muscoïdes	484
„ multipediculosus	485
„ subflavus	447
„ stolonatus	485
Bacterium Zürnianum	487
Bacillus ureæ	487
„ thermophilus	488
'Seidenglänzender bacillus'	489
Bacillus latericeus	439

Bacilli found in Water and known to be Pathogenic to Man or Animals

	PAGE
Spirillum choleræ asiaticæ	399
Vibrio Berolinensis	400
Bacillus typhi abdominalis	410
„ coli communis	411
Bacterium tholœideum	412
„ lactis aërogenes	412
Bacillus anthracis	416
Proteus vulgaris	420
„ mirabilis	420
„ Zenkeri	421
Proteus fluorescens	421
Bacillus tuberculosis	422
„ tetani	423
„ pyocyaneus	424
„ murisepticus	428
„ cuniculicida	429
„ brevis	429
„ capsulatus	430
„ hydrophilus fuscus	431
„ saprogenes II.	432

Micrococci which LIQUEFY *Gelatine*

	PAGE
Staphylococcus pyogenes aureus	498
Micrococcus Biskra	489
'Coccus A' (Foutin)	490
'Rhine-water micrococcus' (Burri)	491
Micrococcus agilis	497
'Grey coccus'	492
Micrococcus candicans	492
„ aërogenes	493
Pediococcus albus	494
Micrococcus fuscus	498
Micrococcus cremoïdes	500
„ flavus liquefaciens	501
„ flavus desidens	502
„ radiatus	503
Sarcina alba	505
„ candida	505
„ lutea	506
„ aurantiaca	507
Streptococcus albus	508
„ vermiformis	508
Diplococcus luteus	509

Micrococci which DO NOT LIQUEFY *Gelatine*

	PAGE
'Coccus B' (Foutin)	490
Micrococcus candidus	493
„ cereus albus	493
„ plumosus	494
„ aquatilis	494
„ violaceus	495
„ cyaneus	495
„ concentricus	495
„ carneus	496
„ cinnabareus	496
„ cerasinus siccus	497
„ citreus	499
Micrococcus luteus	499
„ fervidosus	500
„ aurantiacus	501
„ flavus tardigradus	502
„ rosettaceus	503
„ stellatus	503
„ ureæ	504
„ versicolor	504
„ viticulosus	505
Streptococcus mirabilis	508

Micrococci found in Water and known to be Pathogenic to Man or Animals

	PAGE
Staphylococcus pyogenes aureus	498
Micrococcus Biskra	489
'Coccus B' (Foutin)	490

KOCH'S COMMA SPIRILLUM, OR BACILLUS OF ASIATIC CHOLERA (*Spirillum choleræ asiaticæ*)

LIQUEFIES GELATINE

Authority.—Koch, *Berliner klin. Wochenschrift*, 1884, Nos. 31, 32, 32*a*.

Where Found.—In the dejecta of cholera patients, and in the fresh intestinal contents of cholera corpses. In water.

Microscopic Appearance.—Bent bacilli, frequently hanging together so as to form a semicircle or the letter S. Threads also are formed which give rise to very delicate and long spiral or corkscrew forms. It is very motile, and each rod has a cilium attached to one end; the cilium has generally two distinct bends, and is usually from 1 to 1½ time as long as the whole rod, of which it is about ½ to ⅓ the width. The cilia are stained by adding to 16 c.c. of the mordant from ½ to 1 drop of acid (Loeffler). (See p. 56.) It forms arthrospores according to Hueppe, but it possesses no form which is endowed with any considerable powers of resistance. Numerous involution forms appear in old cultures. They stain best with an aqueous solution of fuchsin. They are not coloured by Gram's method.

Cultures.—

Gelatine Plates.—The colonies are more or less circular, and have a rough irregular surface, with coarse granular contents. Under a low power they are at first light, but become later more opaque in the centre and exhibit short, very fine radial hairy extensions at the periphery. Liquefaction ensues, and each centre is surrounded by a slight funnel-shaped depression.

Gelatine Tubes.—The gelatine is slowly liquefied near the surface in the shape of a funnel; the gelatine at the point of inoculation widens and gives rise to an air-bubble-shaped depression, the lower part of the needle's path in the depth remaining for several days visible as a thin white thread. Ultimately the whole contents of the tube become fluid.

Agar-agar.—Forms a grey-white shining expansion.

Blood Serum.—Grows abundantly, slowly liquefying it.

Potatoes.—It used to be asserted that it would only grow on potatoes at from 30° to 40° C., when it produced a transparent light-greyish brown expansion. Krannhals (see p. 23) has recently shown that this bacillus will grow on slices of potato saturated with a 1 to 2 per cent. solution of sodium carbonate both at 16° to 18° C., as well as at the higher temperature. The bacillus is very sensitive to the slightest trace of free acid, and hence its difficulty in growing on ordinary potato cultures is doubtless due to the presence of acid in such.

Broth.—Produces a wrinkled and much-folded pellicle and a deposit, the liquid itself remaining nearly clear.

Milk.—Sterilised milk is rendered strongly acid, and its coagulation takes place in 48 hours at 37° C. (Haan and Huysse, *Centralb. f. Bakt.*, vol. xv., 1894, p. 268.)

Remarks.—The cultures in media containing peptone give the so-called '*cholera-red*' or nitrosoindol reaction, which is fully described on p. 281. It produces sulphuretted hydrogen in broth cultures (Stagnitta-Balistreri). Finkler-Prior's bacillus resembles Koch's bacillus, but its mode of liquefaction is different, being much more rapid and extending at once to the bottom of the needle's path in the depth, whilst the colonies are smooth-rimmed. (Compare also p. 326, where its behaviour in water is described.) It used to be alleged that the Finkler bacillus was identified with 'cholera nostras,' but this has since been negatived by Finkler. There are many comma-shaped bacilli found in water (see p. 276; see also Koch's remarks on p. 278 on the detection of the cholera bacillus in water). The Comma bacillus grows best at from 30° to 40° C., but below 16° C. its growth appears to cease. It is very sensitive to dry surroundings; thus Rigler (*Centralblatt für Bakteriologie*, vol. xiii., 1893, p. 651) found that when silk threads soaked in fresh broth cultures of the bacillus were simply exposed in the air the bacilli were destroyed in three hours, whilst if similar threads were wrapped up in damp cloths they were still alive after two days. If guinea-pigs are first dosed with tincture of opium and sodium carbonate and then receive an injection of broth cultures of the bacillus in the stomach, they succumb, and the bacilli are found in the intestinal contents and in the mucous membrane of the intestine, and also in the lumina of Lieberkuhn's follicles. Sabolotny (*Centralblatt für Bakteriologie*, vol. xv., 1894, p. 150) states that the marmot is exceedingly sensitive to this bacillus. If 0·1 to 0·2 c.c. of a one-day-old broth culture grown at 37° C. is introduced subcutaneously or into the peritoneum, it dies in from twelve to eighteen hours, and the bacilli are found in the blood, liver, spleen, and in the peritoneal fluid. A small quantity is also fatal when introduced *per os* with food or liquids, previous treatment with soda and opium being unnecessary. For Pfeiffer's method of confirming cholera bacillus by animal experiment see p. 282. For the action of light on this bacillus see p. 386.

VIBRIO BEROLINENSIS

LIQUEFIES GELATINE

Authority.—Neisser, 'Ueber einen neuen Wasser-Vibrio, der die Nitrosoindol-reaction liefert,' *Archiv f. Hygiene*, 1893, p. 194.

Where Found.—In filtered river Spree water.

Microscopic Appearance.—Usually somewhat smaller than the cholera bacillus; otherwise undistinguishable. Very motile; one long and much twisted cilium is attached to one end of the rod. No spore formation observed. Is discoloured by Gram's method.

Cultures.—

Gelatine Plates.—Liquefies the gelatine much more slowly than the cholera bacillus; is often hardly visible on the plate, even after forty-eight hours. Under the microscope at the end of twenty-four hours the depth colonies are small, circular and smooth-rimmed, the contents are very slightly and finely granulated, they are colourless and transparent. The surface colonies form small transparent skin-like expansions, with a central circular disc. No depression is formed in the gelatine, and the edge of the colony always remains sharply defined.

Gelatine Tubes.—In gelatine tubes it is only distinguished from the cholera bacillus by its markedly slower growth.

Agar-agar and Glycerine-agar.—Resembles the cholera bacillus.

Potatoes.—On ordinary potatoes, as well as on those treated with soda, vinegar, and salt respectively, it resembles the cholera bacillus.

Broth.—Renders strongly alkaline broth more rapidly and decidedly turbid at 20° to 22° C. and at 37° C. than the cholera bacillus; otherwise resembles the latter. Grows very luxuriantly and much more so than the cholera bacillus in pancreas broth.

Sterile Water.—At 37° C., after six days no visible growth, but when sterile 1 per cent. pepton-water was added to these tubes, turbidity began forty-eight hours later; the cholera bacillus tube, however, remained clear, no growth having taken place.

Sterile Milk.—No coagulation, and forms no acid; therefore resembles the cholera bacillus.

Remarks.—It will not grow at 10° C., and is destroyed when kept at 60° C. for five minutes. Gives the 'cholera-red' or nitrosoindol reaction. Is pathogenic to guinea-pigs, but not to mice, rabbits, or pigeons.

Note.—Sanarelli ('Les Vibrions des Eaux et l'Etiologie du Choléra,' *Annales de l'Institut Pasteur*, vol. vii., 1893, p. 693) isolated no less than thirty-two different vibrios from the river Seine, drain-water, sewage-effluent, and pond-water, four of which gave the 'cholera-red' or nitrosoindol reaction and were pathogenic to guinea-pigs. Sanarelli is of opinion that there may be numerous vibrios capable of exciting cholera, and that the idea of its propagation being due to one particular variety is untenable. (See pp. 279 and 283.)

VIBRIO AQUATILIS

LIQUEFIES GELATINE

Authority.—Günther, 'Ueber eine neue im Wasser gefundene Komma-bacillenart,' *Deutsche medicinische Wochenschrift*, No. 49, 8 December, 1892, p. 1124. See also Kieszling, 'Ein dem Choleravibrio ähnlicher Komma-bacillus,' *Arbeiten a. d. kaiserlichen Gesundheitsamte*, vol. viii., 1893, p. 430.

Where Found.—Found in river Spree water at Stralau at the intake of the Berlin waterworks. Found by Kieszling in slimy water which had been used for washing the sand used in the water-works at Altona.

Microscopic Appearance.—Resembles in every particular Koch's Comma bacillus (see p. 399). Possesses one cilium at one pole.

Cultures.—

Gelatine Plates.—Forms circular and perfectly smooth-rimmed colonies, brown in colour, and having very fine granular contents, and therefore differs from Koch's Comma bacillus. If near other liquefying colonies on a gelatine plate their edge becomes less sharply defined.

Gelatine Tubes.—Grows exclusively on the surface; the basin-shaped liquid depression, however, gradually extends downwards, but in the needle's path in the depth below the liquid depression practically no growth makes its appearance.

Agar-agar.—Grows like Koch's Comma bacillus both at 15°to 20° C. and at 37° C.

Potatoes.—When tried on four different kinds of potatoes no growth took place, whether at 15° to 20° C. or at 37° C.

Broth.—Refuses to grow in alkaline and neutral broth at 37° C. At 21° to 22° C. hardly any growth takes place, only after some weeks a very slight trace of turbidity made its appearance in both descriptions of broth, which was traced to the growth of the vibrio.

Remarks.—Does not exhibit the 'cholera-red' or nitrosoindol reaction, and is not pathogenic to animals.

BACILLUS CHOLEROIDES *a*

LIQUEFIES GELATINE

Authority.—Bujwid, 'Ueber zwei neue Arten von Spirillen in Wasser,' *Centralblatt f. Bakteriologie*, vol. xiii., 1893, p. 120.

Where Found.—In river water.

Microscopic Appearance.—Resembles Koch's *Comma spirillum*; the movements are, however, not so rapid.

Cultures.—

Gelatine Plates.—Resembles the *Comma spirillum* when grown at 10° to 12° R., but at a higher temperature the colonies are broader and more superficial, and do not sink so deeply into the gelatine, which becomes gradually turbid. Under a low power the contour is more regular than is the case with the *Comma spirillum*; they are almost smooth or very finely granular. Does not give rise to an odour of indol, but recalls that of methyl-mercaptan.

Gelatine Tubes.—Grows on the surface, liquefying only the upper layers. At about 10° to 12° R. the gelatine is more slowly liquefied, and the well-known air-bubble appearance is produced. It hardly grows at all in the depth.

Agar-agar.—Grows luxuriantly in the incubator and gives rise to an odour resembling methyl-mercaptan.

Broth.—Renders it slightly turbid, and no pellicle is produced.

BACILLUS CHOLEROIDES β

LIQUEFIES GELATINE

Authority.—Orlowski. See Bujwid's paper, p. 121.

Where Found.—In well-water in the neighbourhood of which many cholera cases had occurred.

Microscopic Appearance.—This organism resembles the *Comma spirillum* even more closely than the above; it grows, however, more anaërobically, and forms a much deeper liquefying funnel.

Remarks.—Finkelnburg ('Zur Frage der Variabilität der Cholerabacillen,' *Centralblatt f. Bakteriologie*, vol. xiii., 1893, p. 113) states that in consequence of its long residence in culture media the original *Comma bacillus* has undergone a gradual degeneration of its biological energies. It is quite possible, therefore, that the above spirilla may be true cholera organisms, although deviating slightly from Koch's original spirillum.

(EINE NEUE VIBRIONENART)

LIQUEFIES GELATINE

Authority.—Weibel, 'Ueber eine neue im Brunnenwasser gefundene Vibrionenart,' *Centralblatt für Bakteriologie*, vol. xiii., 1893, p. 117.

Where Found.—In a well water.

Microscopic Appearance.—Resembles the *Comma spirillum*, also the *Vibrio saprophiles* α (see p. 404), but on the whole it is rather larger than the former. The most characteristic comma-shaped forms are obtained in broth.

Cultures.—

Gelatine Plates.—Appears at first in the form of dull white dots, which under a low power are translucent light-brown mostly circular discs, with a smooth rim and homogeneous structure. It liquefies the gelatine much more rapidly than the *Comma spirillum*, and under a low power the centre is dark and broken up, surrounded by a light evenly granular outer zone, which is followed by a rather darker zone consisting of closely packed and very fine radial lines. In consequence of the varying degrees of lightness of this border an impression is conveyed of a delicate folding or crinkling of the periphery. Many colonies before liquefaction begins form dull white flat expansions, which under a low power are irregularly circular, pale yellowish in the centre, and dull grey or colourless towards the edge. In these the liquefaction begins in the centre of the colony, the latter forming a circular yellowish depression. The rate of liquefaction is very variable, appearing to depend upon the free access of air, being less rapid in the closed Esmarch tubes than on ordinary plates.

Gelatine Tubes.—Develops all along the needle's path in the depth, forming a flat dish-shaped concavity on the surface; at the bottom of the liquid gelatine a crumbly white deposit forms, whilst the liquid above is quite clear.

Agar-agar.—Forms a grey expansion on the surface; a growth is also visible in the depth. It grows more rapidly at 37° C.

Potatoes.—No development.

Broth.—Renders it slightly turbid, forming a deposit. It, as a rule, forms no pellicle, but a delicate circular growth which clings to the sides of the tube, and which is easily disengaged by shaking, and sinks often without breaking to the bottom of the tube. At 37° C. the development is more rapid and luxuriant.

Remarks.—When exposed to 55° C. for thirty minutes it is destroyed.

PSEUDO-CHOLERA SPIRILLUM (Rénon)

LIQUEFIES GELATINE

Authority.—Rénon, 'Etude sur quatre Cas de Choléra,' *Annales de l'Institut Pasteur*,' vol. vi., 1892, p. 621.

Where Found.—In well water at Billancourt, in the vicinity of Paris. The well, which was highly polluted, was near the Seine, and the water in it was at the same level as that of the river.

Microscopic Appearance.—A spirillum three to four times as long and two to three times as broad as Koch's cholera spirillum; gives rise also, however, to 'S' forms.

Cultures.—

Gelatine Plates.—Under a low power after three days the colonies are small and lenticular, with rounded edges and very fine peripheral extensions; the centre is darker and yellowish in colour. On the fourth day the colony becomes surrounded by a liquid zone, and after the sixth day no longer increases in size.

Gelatine Tubes.—Liquefaction commences on the second day, and the upper part of the tube becomes filled with an air bubble, immediately beneath which is seen a growth about a millimetre in thickness due to the accumulation of spirilla, whilst at the lower portion of the needle's track in the depth are seen colonies arranged so as to resemble a twisted fringe. The whole contents of the tube become fluid in from ten to twelve days. The growth much resembles that of Koch's Comma spirillum, but is more rapid.

Agar-agar.—At 37° C., at the end of ten hours a thick creamy-white growth about a millimetre broad appears along the needle-streak.

Broth.—At 37° C. the liquid becomes turbid in six hours, and a considerable deposit has collected by the next day. On the third day a very thin pellicle makes its appearance.

Remarks.—It is not pathogenic to guinea-pigs. Two people who had drunk this well-water were seized with cholera, and Koch's cholera spirillum was separated out from the intestinal contents and the stools of one of the victims. The water itself was not collected for examination until fifteen days after the death of this patient, and, although investigated on the day following its collection, no cholera organisms could be found. Doubtless during the interval, and in the absence of any fresh access of cholera germs to the well, the latter had become outnumbered by other microbes present, 'enormous numbers' of *B. coli-communis* being specially mentioned as present along with the above pseudo-cholera spirillum.

VIBRIO SAPROPHILES *a*

Authority.—Weibel, 'Untersuchungen über Vibrionen,' *Centralblatt f. Bakteriologie*, vol. ii., 1887, p. 469.

Where Found.—In putrid hay infusion, also in sewer mud.

Microscopic Appearance.—Forms bent rods about 3 μ long; in the middle the width is about one-fifth that of the length, for it frequently becomes narrower at the ends. Frequently 'S' forms are seen, but it rarely forms long filaments. In broth and agar-agar cultures about eight days old delicate spiral forms are found. It is very motile.

Cultures.—

Gelatine Plates.—Grows slowly. Under a low power in transmitted light the depth colonies are smooth-rimmed, yellowish-brown, circular discs; the centre is dark, surrounded by concentric rings. Later the rim becomes serrated. The surface colonies are flat yellowish-white expansions; under a low power they are not circular, and exhibit a sharply defined dark centre, whilst towards the periphery the yellowish-grey colour becomes paler; the contents are finely granular. No liquefaction takes place.

Gelatine Tubes.—Forms a veil-like streak in the depth and a whitish expansion on the surface; the gelatine which is not occupied by the growth looks as if it had been covered by a transparent whitish cloud.

Agar-agar.—Does not grow in the depth, but forms an abundant dirty yellowish-white spreading expansion, beneath which the agar-agar is clouded for a distance of 1 to 2 mm.

Potatoes.—Forms in two days an abundant slimy pasty growth, yellowish-red in colour, which becomes darker until it is a full chocolate-brown.

Broth.—Renders the liquid turbid, and produces a yellowish crumbly deposit, in which wavy twisted threads are found.

Remarks.—It retains its vitality over a considerable period in agar-agar cultures, a nine months old cultivation yielding growths on being re-inoculated (Weibel, *Centralblatt f. Bakteriologie*, vol. iv., 1888, p. 230).

VIBRIO SAPROPHILES γ

Authority.—Weibel, 'Untersuchungen über Vibrionen,' *Centralblatt f. Bakteriologie*, vol. iv., 1888, p. 231.

Where Found.—In sewer mud.

Microscopic Appearance.—Resembles the *V. saprophiles* α, but is about twice as large. It rarely forms long twisted threads. It has a great tendency to produce involution forms. It is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—The depth colonies are white and under a low power they are ovoid, smooth-rimmed and granular. The centre is orange-coloured and sharply defined, and surrounded by a light yellow zone. The colour becomes darker with age. The surface colonies are flat, dirty white, somewhat opalescent expansions, with a prominent white centre. Under a low power they resemble to a certain extent the *B. coli-communis*. The edge is irregular and lobular; the zone nearest the periphery is pure white, penetrated by numerous very fine ovoid furrows, upon which follows a light yellow ochre zone, which is streaked with darker washed out spotted stripes. The centre is yellowish brown, containing also delicate dark wavy lines. Later the peripheral zone becomes yellowish, and the remaining zones decidedly darker. No liquefaction takes place.

Gelatine Tubes.—Forms a streak in the depth and a moderate-sized whitish expansion on the surface.

Agar-agar.—No development in the depth, but forms a dirty-white pasty expansion covering the whole surface.

Potatoes.—Very inconstant in its growth, being sometimes a dark brown growth, very dry and tough, resembling a mould, and at others yellowish brown, moist and shining, often also mahogany brown in colour.

Broth.—Renders it turbid and produces a thick firm pellicle, which sinks to the bottom when shaken, but forms again later.

VIBRIO AUREUS

Authority.—Weibel, 'Untersuchungen über Vibrionen,' *Centralblatt f. Bakteriologie*, vol. iv., 1888, pp. 225, 257 and 289.

Where Found.—In sewer mud.

Microscopic Appearance.—About 1½ time as thick as the Comma spirillum. Typical comma and 'S' forms visible, with rather plump and blunted ends; also gives rise to longer and shorter forms, the latter being sometimes even oval. The tendency to become spiral appears to be less pronounced than in most spirilla Degenerative forms are also found. It is not motile. It is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—Grows in the depth, and on the surface forms flat expansions of a pure yellow-gold colour. Under a low power the surface colonies are circular, smooth-rimmed, granular, golden yellow in the centre, and paler towards the edge. The depth colonies are ovoid and rather coarsely granular; the centre is golden yellow to start with, and the periphery lighter. Later on a spindle-shaped thickened portion appears in the centre, which later becomes brown and quite black, whilst on both sides the golden yellow edge is visible. No liquefaction ensues.

Gelatine Tubes.—Forms a rather thick finely granular growth along the needle's path in the depth. It forms a round bowl-shaped expansion on the surface. Both in the depth and on the surface the colour is yellow ochre.

Agar-agar.—Grows only on the surface, forming a dirty white expansion in which raised spots of a yellow colour become visible; finally the growth becomes 2 mm. thick, and forms a uniform pasty covering of a golden yellow colour.

Potatoes.—Grows abundantly, forming a thick pasty golden yellow expansion.

Broth.—Renders the liquid turbid and forms a deposit, but no pellicle.

VIBRIO FLAVUS

Authority.—Weibel, 'Untersuchungen über Vibrionen,' *Centralblatt f. Bakteriologie*, vol. iv., 1888, p. 260.

Where Found.—In sewer mud.

Microscopic Appearance.—Resembles *Vibrio aureus*.

Cultures.—

Gelatine Plates.—The shape of the colonies resembles *Vibrio aureus*; the colour on a dark ground is dirty greyish yellow, on a white ground straw yellow. Under a low power the depth colonies are golden yellow, but with no dark centre, and are very finely granular. The surface colonies are pale yellow, with dull grey washed-out spots; the periphery has usually a white zone. No liquefaction ensues.

Gelatine Tubes.—Resembles *Vibrio aureus*.

Agar-agar.—Resembles *Vibrio aureus*, only produces a yellow ochre colour.

Potatoes.—Resembles *Vibrio aureus*, only produces a yellow ochre colour.

Broth.—Resembles *Vibrio aureus*.

VIBRIO FLAVESCENS

Authority.—Weibel, 'Untersuchungen über Vibrionen,' *Centralblatt f. Bakteriologie*, vol. iv., 1888, p. 260.

Where Found.—In sewer mud.

Microscopic Appearance.—Resembles *Vibrio aureus*.

Cultures.—

Gelatine Plates.—The shape of the colonies resembles *Vibrio aureus*; on a dark ground the colour is dirty greyish yellow, on a light ground dirty greenish yellow, forming a ready mark of distinction between the colonies of *Vibrio aureus* and *Vibrio flavus*. Under a low power the colonies resemble *Vibrio aureus*, but the shade of yellow is lighter, feebler, and less pure. No liquefaction ensues.

Gelatine Tubes.—Resembles *Vibrio aureus*, the expansion being, however, instead of bowl-shaped, flatter, with a lobular edge.

Agar-agar.—Resembles *Vibrio aureus*, the colour being, however, dull yellow, with isolated grey-coloured spots.

Potatoes.—Resembles *Vibrio aureus*, the colour being, however, dull yellow.

Broth.—Resembles *Vibrio aureus*.

SPIRILLUM RUBRUM

Authority.—Esmarch, 'Ueber die Reincultur eines Spirillum,' *Centralblatt f. Bakteriologie*, vol. i., 1887, p. 225.

Where Found.—In water in which the body of a mouse dead of septicæmia had been allowed to putrefy. Found by Adametz in water.

Microscopic Appearance.—Forms short spirilla with 1, 2, or 3 screw twists in gelatine, agar-agar, and potatoes, but as many as 30, 40, and 50 in broth cultures. It is about twice as thick as the Comma spirillum. The shorter spirilla are very motile, the longer ones are either motionless or capable of very slight motility. Shining spots are visible in the interior, which, although refusing to stain like spores, are regarded as such.

Cultures.—

Gelatine Plates.—Grows exceedingly slowly, eight days often elapsing before any colony becomes visible to the naked eye. It forms grey or pale red centres, with somewhat granular contents and a very nearly smooth rim; gradually the colour becomes wine-red, especially the deeper down the colonies are in the depth. No liquefaction takes place.

Gelatine Tubes.—Grows the whole length of the needle track in the depth, forming closely compressed round wine-red colonies, but on the surface and near the surface, where air has access, it produces no coloured growth.

Agar-agar and Blood Serum.—Forms a moist, shining, grey-white restricted expansion, becoming wine-red in the thicker parts of the growth. The condensed water becomes filled with very long spirilla, and later a red sediment collects.

Potatoes.—Grows very slowly, the deep red colonies never exceeding in size that of a hemp-seed.

Broth.—Forms a red sediment and renders the liquid slightly turbid.

Remarks.—Not pathogenic when subcutaneously introduced into mice, guinea-pigs and rabbits. It is killed when exposed to 42° C. for twenty-four hours; grows best at 37° C.

SPIRILLUM RUGULA

LIQUEFIES GELATINE

Authority.—Müller, Vignal, *Archives de Physiologie*, vol. xviii. *b*, p. 333.

Where Found.—In the buccal cavity; also in stagnant water and putrefying liquids.

Microscopic Appearance.—Rods 6 to 8 μ long and 0·2 to 2·5 μ broad, simply bent or forming a flat spiral twist, sometimes in longer chains. Cilia are found at the poles. It has a lively rotatory movement. Forms round spores at the end of the rod. (Prazmowski.)

Cultures.—Can only be cultivated in the absence of air.

Gelatine Plates.—At 20° to 22° C. it forms yellowish-white ball-shaped discs; later liquefaction takes place.

Gelatine Tubes.—At 20° to 22° C. forms small white pin-head growths on the surface and along the needle's path in the depth. Later liquefaction takes place.

Agar-agar.—At 36° to 38° C. forms a white and slightly folded expansion.

Potatoes.—At 36° to 38° C. forms a white wrinkled expansion, which rapidly spreads over the whole surface, and in older cultures assumes a yellowish colour.

Blood Serum.—At 36° to 38° C. it grows rapidly, liquefying the serum and forming a white pellicle.

Remarks.—It is anaërobic. Gives rise in all culture media to a very penetrating fæcal odour.

SPIRILLUM MARINUM

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bakterien,' *Zeitschrift für Hygiene*, vol. xi., 1891, p. 198.

Where Found.—Occasionally in sea water and sea mud.

Microscopic Appearance.—Small bacillus usually in pairs, more or less bent, although straight individuals are also found. When several join together to form a filament, the whole assumes the characteristic spirilla form. It is capable of rotatory as well as progressive movements, and moves rapidly with a corkscrew-like motion across the microscopic field. No spore formation was observed. Stains easily with Loeffler's blue and also with fuchsin, becoming very deeply coloured with the latter.

Cultures.—

Gelatine Plates.—Forms round small radially striped granular masses, but when liquefaction of the gelatine begins the colony becomes rougher in appearance, and flocculent particles float about in the shallow liquid depression.

Gelatine Tubes.—Grows rapidly, liquefying the gelatine and rendering it turbid, and forming a thin semi-transparent pellicle on the surface.

Sea-water Gelatine Tubes.—The growth is more restricted, and a thick pellicle forms on the surface of the shallow liquid depression.

Agar-agar.—Grows abundantly, forming a moist liquid whitish expansion, resembling pus in appearance. This collects at the bottom of the tube, and becomes later watery.

Potatoes.—In twenty-four hours a reddish brown sharply circumscribed expansion appears, which increases in size and forms a thick wax-like mass, which later covers nearly the whole potato, but remains soft and entirely superficial, although the potato itself becomes gradually of a dark greyish green colour.

Sea-water Broth.—Grows well, rendering the liquid very turbid, forming an abundant and fine deposit and a white smooth pellicle on the surface. Ordinary broth is rendered less turbid.

Remarks.—It will not grow at 37° C.

SPIRILLUM CONCENTRICUM

Authority.—Kitasato, 'Ueber die Reincultur eines Spirillum aus faulendem Blute,' *Centralblatt f. Bakteriologie*, vol. iii., 1888, p. 73.

Where Found.—In putrid blood. Included by Lustig (*loc. cit.*) amongst organisms found in water.

Microscopic Appearance.—Small screw-shaped forms with 2 to 3 twists, and with pointed ends. In broth cultures they form long screws with 5 to 20 twists. The diameter of the screw is 2·0 to 2·5 μ, and the length of one twist 3·5 to 4 μ. In thickness it slightly exceeds the *Comma spirillum*. It is very motile, with a screw-like movement. No spore formation observed.

Cultures.—

Gelatine Plates.—Appears in transmitted light as pale grey circular disc composed of concentric cocade-like rings. The centre is whitish and opaque; then follows a transparent ring, then an opaque grey white circle double as broad, then a very narrow transparent ring, and finally a broadish greyish white band, from which are seen extending (under a low power) numerous small twisted prolongations. No liquefaction takes place.

Gelatine Tubes.—It grows more on the surface than in the depth, and after several weeks the whole surface of the gelatine is covered with a cloud-like expansion.

Agar-agar.—Forms a diffused expansion over the surface which adheres so firmly to the agar-agar that it is almost impossible to get any of the growth without bits of agar.

Potatoes.—It does not grow either at the ordinary temperature or at 37° C.

Broth.—Renders it slowly turbid. In very old cultures an abundant slimy deposit collects, whilst the liquid remains clear.

Remarks.—Not pathogenic to mice, guinea-pigs, or rabbits.

SPIRILLUM VOLUTANS (Ehrenberg)

Where Found.—In stagnant water and especially in marsh-water. (Lustig, *loc. cit.*)

Microscopic Appearance.—Filaments 25 to 30 μ long and from 1·5 to 2 μ broad, the ends slightly thinner and rounded, having 2 to 4 spiral turns wide apart. Each filament has a cilium. In the interior of the protoplasm numerous dark granulations are visible, considered by some observers to be sulphur. It is very motile, although according to Lustig the filaments are sometimes motionless.

SPIRILLUM LEUCOMELAENUM

Authority.—Perty, *Zur Kenntniss kleinster Lebensformen*. Berne, 1852.

Where Found.—In stagnant water.

Microscopic Appearance.—Short individuals joined on end to end and forming spirilla with 2 to 3 spiral turns. The contents are deep black, surrounded by a clear aureole.

SPIRILLUM AMYLIFERUM (Van Tieghem)

Where Found.—In water.

Microscopic Appearance.—Rigid filaments rolled up towards the right, 6 μ long and from 1 to 1·5 μ broad, with from 2 to 4 spiral turns. It multiplies by division and spores. In the absence of air it acts as a vigorous ferment.

SPIRILLUM PLICATILE (Ehrenberg)

Authority.—Koch, *Cohn's Beiträge zur Biologie d. Pflanzen*, vol. ii.

Where Found.—In stagnant waters, especially those containing living or dead plants, or decomposing organic matter.

Microscopic Appearance.—Very thin filaments with narrow spiral twists, reaching sometimes 100 to 200 μ in length, and 0·5 μ broad, with rounded ends. It is very motile.

SPIRILLUM RUFUM

Authority.—Perty, *Zur Kenntniss kleinster Lebensformen.* Berne, 1852.

Where Found.—In well-water. It forms on the surface of the sides of the well red mucous spots varying in colour from rose-red to blood-red. (Roux, *loc. cit.*)

Microscopic Appearance.—Forms filaments from 8 to 16 μ long, slightly reddish, with $1\frac{1}{2}$ to 4 spiral turns. It is very motile. It is not broken up into segments.

SPIRILLUM SERPENS (Müller)

Where Found.—In stagnant water and putrefying liquids.

Microscopic Appearance.—Thin filaments, often joined together in chains from 11 to 28 μ long and from 0·8 to 1 μ broad, with 3 to 4 flattened spiral turns. It is very motile. Sometimes it occurs in superficial flakes or pellicles, in which it is bound together by mucous matter.

SPIRILLUM TENUE (Ehrenberg)

Where Found.—In stagnant water.

Microscopic Appearance.—Filaments from 4 to 15 μ in length, and about 0·4 μ broad, having 1 to 5 spiral turns very far apart. It is very motile.

SPIRILLUM UNDULA (Müller)

Where Found.—Frequent in stagnant putrid water.

Microscopic Appearance.—Filaments 8 to 16 μ in length, and about 1 to 1·5 μ broad, having 1 to 6 spiral turns. It is very motile, and has cilia. Forms frequently coarse, mucous flakes.

TYPHOID BACILLUS

(*Bacillus typhi abdominalis*)

Authority.—Eberth, *Virchow's Archiv*, vol. lxxxi., 1880; also *ibid.* vol. lxxxiii., 1881. Gaffky, 'Zur Aetiologie des Abdominaltyphus,' *Mittheilungen a. d. kaiserlichen Gesundheitsamte*, vol. ii., 1884, p. 372.

Where Found.—In the blood, urine, fæces, as well as in the organs of typhoid patients. Found by numerous investigators in water.

Microscopic Appearance.—A short, plump bacillus about three times as long as broad, with rounded ends. It occurs in the tissues usually singly, but in artificial cultures it grows frequently into long threads. It is very motile and is provided with numerous cilia, which are attached to both the sides and ends of the bacillus. To stain the cilia add 22 drops of caustic soda to 16 c.c. of the mordant (Loeffler) (see p. 56). It is not stained by Gram's method, and stains less readily with aqueous aniline solutions than most bacteria. Günther recommends heating the cover-glass, after the dye has been poured on it, for a few seconds until it begins to steam, and then washing off the stain as usual. It does not form spores.

Cultures.—

Gelatine Plates.—The colonies on the surface form large spreading greyish white iridescent expansions with jagged and irregular edge. Under a low power they exhibit a brownish shimmer and a characteristic woven structure. The depth colonies are darker, with regular edge, and are covered with delicate irregular lines. No liquefaction takes place.

Gelatine Tubes.—Grows chiefly on the surface, producing a delicate greyish white iridescent expansion with irregular edge.

Agar-agar.—Forms a greyish white moist expansion.

Potatoes.—Produces an almost invisible greyish white growth after forty-eight hours, but on touching the moist-looking surface with the needle a tough resistant pellicle is found. On different potatoes, however, its growth is more apparent, so that the above is not the only appearance to which it gives rise.

Blood Serum.—Produces a milk-white expansion restricted to the path of the needle.

Broth.—Renders it turbid.

Milk.—Grows abundantly, rendering it slightly acid. No coagulation takes place.

Remarks.—It grows best at about 37° C. Kitasato states that it produces no indol reaction (see p. 273). It produces sulphuretted hydrogen in iron-gelatine (see p. 14), the needle-track after from five to six days becoming intensely black in colour. In iron-agar, at from 33° to 34° C., this black colour appears at the end of twenty-four hours (Fromme). It produces sulphuretted hydrogen in broth with or without peptone; comparative tests made with the *B. coli-communis* revealed no difference either in the degree of the reaction (as shown by the lead-paper test) or in the rapidity with which it took place in the case of these two organisms. The typhoid bacillus never produces gas in any artificial media (see p. 269). It is destroyed when heated for ten minutes at 60° C. Injection into the aural vein of rabbits causes death in twenty-four to twenty-eight hours (Fraenkel and Simmonds); guinea-pigs into which the cultures are introduced by the mouth, as described for cholera, are also killed (Seitz). Opinion is, however, still divided as to whether death is due to mere intoxication by the bacterial products present in the cultures or to actual multiplication of the bacillus within the animal. In this connection see Petruschky (*Zeitschr. f. Hygiene*, vol. xii., 1892, p. 269). For the effect of insolation on this bacillus see Chapter IX. It will not grow in formalin-broth (1 : 7000), see p. 285.

BACILLUS COLI-COMMUNIS

Authority.— Escherich, *Fortschritte der Medicin*, vol. iii., 1885, Nos. 16 and 17. Also Dunbar, 'Ueber den Typhus-bacillus und den Bacillus coli-communis,' *Zeitschrift f. Hygiene*, vol. xii., 1892, p. 485. Also Luksch, 'Zur Differenzialdiagnose des Bacillus typhi abdominalis (Eberth) und des Bacterium Coli-commune (Escherich),' *Centralblatt f. Bakteriologie*, vol. xii., 1892, p. 427.

Where Found.—In the intestinal tract of man and animals. Found in the urine in cases of cystitis by Reymond, *Annales des Organes Génito-Urinaires*, Paris, vol. xi., No. 10, p. 734. Found often in water by numerous investigators, and frequently mistaken for the typhoid bacillus.

Microscopic Appearance.—The typical form is a short bacillus 0·4 μ broad and 2 to 3 μ long; it is, however, very variable, oval individuals and forms resembling cocci being also found. It exists chiefly as a double bacillus arranged in groups. It is slightly motile, and is provided with 1 to 3 cilia, whilst the typhoid bacillus has 8 to 12 cilia (Luksch). Nicolle and Morax mention that the Coli bacillus has invariably fewer cilia than the typhoid, that whereas the former rarely possesses more than 6, the latter usually exhibits 10 to 12, whilst the cilia of the former are also far more fragile (*Annales de l'Institut Pasteur*, vol. xii., 1893, p. 561). It does not form spores.

Cultures.—

Gelatine Plates.—Forms round and very often oval smooth-rimmed granular colonies in the depth, which later become yellowish brown in colour. On the surface it forms flat irregular pale white expansions, which under a low power exhibit a furrowed appearance due to the unequal thickness of the colony in its different parts. The colony also presents a distinctly wavy lineal structure parallel to the periphery. No liquefaction ensues.

Gelatine Tubes.—Grows somewhat abundantly in the depth, producing small white pin-head colonies, whilst on the surface it forms an expansion resembling the growth on gelatine plates.

Agar-agar.—Grows abundantly on the surface, producing a dirty white faintly shining expansion.

Blood Serum.—Forms a milk-white expansion.

Potatoes.—Produces a slimy yellow expansion on some potatoes, on others grey white, whilst in some cases it resembles the typhoid bacillus in being hardly visible.

Broth.—Renders it turbid.

Milk.—Renders it acid, and at 37° C. coagulates it in from twenty-four to forty-eight hours.

Remarks.—Broth cultures of twenty-four hours' age generally exhibit considerable evolution of gas; ordinary gelatine or agar stab-cultures also generally exhibit bubbles of gas in the solid medium. Such bubbles can invariably be obtained by inoculating into ordinary melted gelatine, which is afterwards allowed to solidify (Percy Frankland). The addition of dextrose to the gelatine is quite unnecessary for this purpose. Exhibits indol reaction (see p. 273) after twenty-four to forty-eight hours' culture in peptone broth. Is capable of exhibiting very different degrees of pathogeneity according to its origin; cultures made from diseased tissues in which it is present on being intraperitoneally inoculated into rabbits causes peritonitis, and the bacilli are found in pure culture in the heart's blood. (Alex. Fraenkel, *Wiener klin. Wochenschr.*, 1891, Nov. 13–15.) Grows in formalin-broth (1 : 7000), see p. 285.

BACTERIUM THOLOEIDEUM

Authority.—Gessner, *Archiv für Hygiene*, vol. ix. p. 129.

Where Found.—In the intestinal tract of healthy people. In water suspected of causing typhoid fever by Schardinger, *Wien. klin. Wochenschrift* V., Nos. 28, 29. (*Centralblatt f. Bakteriologie*, vol. xv. p. 48.)

Microscopic Appearance.—Short rods with rounded ends, also in oval forms. Resembles *B. lactis aërogenes* (see below), also in its mode of growth.

Cultures.—

Gelatine Plates.—On the surface the colonies form at first nail-head growths of slimy, stringy consistency, and of an opaque, dirty white colour; later they lose their slimy character, and form large circular expansions, with a grey centre surrounded by concentric grey rings. Under a low power they are circular and smooth-rimmed, with a colourless, bright and shining periphery. From the centre brownish yellow very fine radial lines extend, but they become yellow as they approach the periphery. Later the centre becomes greyish brown and the remainder grey, whilst the bright peripheral zone disappears. The depth colonies are whetstone-shaped and are of a yellowish white colour. Under a low power they are at first olive green in colour, later dark grey-green, and then resemble date stones in shape.

Gelatine Tubes.—Forms a moist, shining convex expansion, which later becomes thick and spreads over the whole surface. In the depth it forms a yellowish white thick band with closely packed small round extensions, having button-shaped and thick ends.

Agar-agar.—Forms a moist thick expansion.

Potatoes.—In from two to three days forms a shining, yellowish expansion, which rapidly spreads over the whole surface and has a slightly lobular and sharply defined edge. On one occasion small bubbles of gas were found on a potato culture four days old.

Remarks.—Grows at the ordinary temperature. It is pathogenic to mice (thus distinguished from the *B. lactis aërogenes*) and guinea-pigs. The bacteria are found in the blood and organs of the body, and are easily isolated and cultivated.

BACTERIUM LACTIS AËROGENES

Authority.—Escherich, 'Die Darmbakterien des Säuglings u. ihre Beziehung z. Physiol. d. Verd.,' 1886, *Fortschr. der Medicin*, 1885, No. 15.

Where Found.—In the intestinal tract of animals and people fed with milk, especially in that of suckling children and animals. Found also once in unboiled cow's milk. Found in urine in cases of pneumaturia, by Heyse (*Centralbl. f. Bakt.*, vol. xv., 1894, p. 322). Found by Schardinger (*loc. cit.*) in water suspected of causing typhoid fever.

Microscopic Appearance.—Short plump rods 0·5 to 0·8 μ broad and 1 to 2 μ long, with rounded ends. Usually arranged in pairs side by side; also found grouped together in irregular heaps. It is not motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms on the surface convex isodiametric moist shining porcelain-white colonies; in the depth yellowish round centres. No liquefaction ensues.

Gelatine Tubes.—Grows abundantly along the needle's path in the depth, and produces a nail-head shaped expansion on the surface.

Potatoes.—Produces vigorous white centres impregnated with bubbles of gas. The colonies sometimes run together and resemble cream.

Blood Serum.—Forms a raised, moist, shining, white expansion.

Remarks.—When subcutaneously introduced into rabbits and guinea-pigs, these animals, especially the latter, die in from one to three days, and the bacilli are found in the blood and organs. It is not pathogenic to mice. It grows best at the temperature of the body. It is aërobic, and facultatively anaërobic in milk, sugar, and grape sugar solutions; in these media when thus grown it produces gas consisting of carbonic anhydride and hydrogen.

BACILLUS AQUATILIS SULCATUS I.

Authority.—Weichselbaum, *Das österreichische Sanitätswesen*, 1889, Nos. 14 to 23.

Where Found.—In the Vienna 'Hochquellenleitungswasser' at the time of the introduction of the water obtained from the Schwarza, a little river in the vicinity of the Sœmmering.

Microscopic Appearance.—Small rods resembling the typhoid bacilli. Is very motile. No spores observed. Is not stained by Gram's method.

Cultures.—

Gelatine Plates.—Circular superficial colonies appear on the plates after two days, the periphery of which is very thin and bluish in colour, whilst the centre is thicker and whitish. The edge is distinctly serrated. Under a low power the surface is seen to consist of numerous fine lines crossing one another from various parts, resembling the typhoid colonies. The edge of the colony is white and the centre yellowish. Later on the lines in the centre become much more marked and intricate, whilst the periphery remains white and of the same appearance as before, but after four days the whole colony becomes yellow. No liquefaction of the gelatine takes place.

Gelatine Tubes.—Exhibits a flat white expansion on the surface, with serrated edge, after twenty-four hours; it looks thicker than in the typhoid growth, and is more restricted than the latter.

Agar-agar.—Produces at 37·5° C. a somewhat thick white expansion, having an odour of whey.

Potatoes.—No growth is visible at 37·5° C., the point of inoculation only has a moist appearance. At the temperature of a room this is also the case at first, but later a very thin, shining and moist expansion of a cream colour becomes visible. The potato in the vicinity of the growth sometimes becomes of a blue grey colour.

Remarks.—It grows far more rapidly than the typhoid bacillus at the temperature of the room, but only develops scantily at 37° C. It produces a visible growth at from 5° to 7° C., at which temperature the typhoid bacillus cannot develop.

BACILLUS AQUATILIS SULCATUS II.

Authority.—Weichselbaum, *Das österreichische Sanitätswesen*, 1889, Nos. 14 to 23.

Where Found—In the Vienna 'Hochquellenleitungswasser' at the time when the water from the river Schwarza was introduced. Also by Tataroff in Dorpat well water (*Die Dorpater Wasserbacterien*, 1891, p. 31).

Microscopic Appearance.—Short rods with rounded ends resembling the smaller typhoid bacilli. Is motile. No spore-formation observed. It is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—The surface colonies resemble at the end of two days those of the typhoid bacillus and the *B. aquatilis sulcatus* I., but they are somewhat thicker and the edge is not visibly serrated. With a low power the periphery is seen to be slightly serrated, but the system of lines is not so clearly defined as in the *B. aquat. sul.* I. The centre is yellow and the periphery white. At the end of three days the colonies have become thicker and no signs of lines or serration are visible under the microscope, and the colonies appear, with exception of the outer zone, quite yellow in colour.

Gelatine Tubes.—Rather thicker and more restricted; otherwise like *B. aquat. sulc.* I.

Broth.—The liquid becomes turbid in from twenty-eight to thirty-seven hours; a deposit is formed at the end of two days.

Agar-agar.—At 37° C. a grey white expansion is visible in twenty-four hours.

Potatoes.—At room-temperature the point of inoculation at first exhibits a yellow blue colour, but later a yellow grey or yellow brown expansion makes its appearance, which is at times very luxuriant. A faint smell of urine attends its growth.

Remarks.—In respect of temperature it resembles *B. aquatilis sulcatus* I.

BACILLUS AQUATILIS SULCATUS III.

Authority. — Weichselbaum, *Das österreichische Sanitätswesen*, 1889, Nos. 14 to 23.

Where Found.—In the Vienna 'Hochquellenleitungswasser' at the time when the water from the river Schwarza was introduced.

Microscopic Appearance.—Very short rods, often resembling cocci. Is very motile. No spores observed. Is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—The surface colonies are visible in from two to three days as fine discs, thicker in the centre, which is white, than at the periphery which is serrated, very thin and bluish in colour. Under a low power the system of lines is very clearly defined. As they become older, the colonies become larger and thicker, lose their bluish tint, and the lines are replaced by a much closer medley of short lines and furrows, whilst the colonies assume a yellowish colour. There is nothing characteristic about the depth colonies.

Gelatine Tubes.—Forms in twenty-four hours a very thin white expansion with serrated edge, which rapidly reaches the walls of the tube. It emits a very disagreeable smell. The gelatine is not liquefied.

Agar-agar.—Forms at 37° C. an abundant grey white expansion, which smells like serum.

Broth.—The cultures have a most unpleasant odour.

Potatoes.—At room-temperature it produces an abundant growth, bright yellow in colour. The edge of the expansion is irregular in contour. At the end of nine days the whole potato in the vicinity of the growth assumes a bluish green colour.

Remarks.—In respect of temperature it resembles *B. aquatilis sulcatus* I.

BACILLUS AQUATILIS SULCATUS IV.

Authority.—Weichselbaum, *Das österreichische Sanitätswesen*, 1889, Nos. 14 to 23.

Where Found.—In the Vienna 'Hochquellenleitungswasser' at the time when the water from the river Schwarza was introduced.

Microscopic Appearance.—The length of the individual bacilli varies according to the medium in which they are cultivated. In broth they give rise to threads. The shorter bacilli are motile, but not the threads. No spore formation observed. They are not stained by Gram's method.

Cultures.—

Gelatine Plates.—The surface colonies are visible on the fourth day, and are at first very thin and of a bluish colour with serrated edge and a white and rather thicker centre. Under a low power the characteristic arrangement of lines previously mentioned is visible, the smaller colonies are white and the larger ones yellow in the centre. Later the whole colony becomes of a yellow colour and looks larger and thicker, whilst in the place of the lines irregular furrows make their appearance. The depth colonies are round and yellow. The gelatine is not liquefied.

Gelatine Tubes.—Grows slowly; later a somewhat thin grey white expansion, with serrated edge, gradually extends over the gelatine surface up to the walls of the tube.

Agar-agar.—At room-temperature gives rise after two days to a grey white expansion; at 37° C., however, it grows with difficulty, even after six days there being only a scanty development.

Potatoes.—No growth.

BACILLUS AQUATILIS SULCATUS V.

Authority. — Weichselbaum, *Das österreichische Sanitätswesen*, 1889, Nos. 14 to 23.

Where Found.—In Vienna 'Hochquellenleitungswasser' at the time when the water from the river Schwarza was introduced.

Microscopic Appearance.—The individual bacilli vary in size, are somewhat thicker than the typhoid bacillus, and have rounded and also pointed ends. It is motile. No spore formation observed. It is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—Resembles *B. aquatilis sulcatus* I.

Gelatine Tubes.—A somewhat thin grey white expansion appears after two days, which increases in size, and becomes later of an egg-yolk yellow colour. The gelatine is not liquefied.

Agar-agar.—It will only grow at room-temperature, when it exhibits an abundant yellow viscid growth.

Broth.—Grows at room-temperature, producing a white deposit.

Potatoes.—Produces at room-temperature a pale yellow growth, whilst in the vicinity the potato becomes a dark grey colour. The latter disappears later, and the growth becomes honey-coloured.

BACILLUS ANTHRACIS

LIQUEFIES GELATINE

Authority.—Rayer and Davaine, *Bulletin de la Société de Biologie de Paris*, 1850. Pollender, *Vierteljahrschr. f. ges. Med.*, vol. viii., 1855. Pasteur and Joubert, *Comptes rendus*, 1877. Koch, *Cohn's Beiträge z. Biologie d. Pflanzen*, vol. ii. Heft. 2, 1877; also 'Zur Aetiologie des Milzbrandes,' *Mittheilungen a.d. kaiserlichen Gesundheitsamte*, vol. i., 1881, p. 49.

Where Found.—In the blood of animals dead of anthrax. Diatroptoff (*Annales de l'Institut Pasteur*, vol. vii., 1893, p. 286) found this bacillus in the sediment at the bottom of a well, the water from which had communicated anthrax to a flock of sheep.

Microscopic Appearance.—The bacillus is 1 to 1·5 μ broad, and 3 to 6 to 10 μ long, with square cut ends. Forms long threads in broth at 36° C. It is not motile. Forms spores between 18° and about 40° C.; at 37° they are produced in about twenty-four hours, at 21° C. in about seventy-two hours. The spores appear in the middle of the rod and are only produced in the presence of oxygen, and hence are never formed in the body of an animal. They are stained best by means of Ehrlich's aniline fuchsin solution (see p. 45). The bacilli stain readily with the usual aqueous solutions.

Cultures.—

Gelatine Plates.—Under a low power the depth colonies are circular and consist of twisted and knotted bands of threads closely packed together, which give an irregular appearance to the edge. On the surface the colonies consist of masses of convoluted threads extending in all directions and producing the most varied appearance, sometimes recalling a medusa head, whilst colonies consisting entirely of banded threads with long curled whip-like projections are also found. The gelatine is liquefied.

Gelatine Tubes.—The gelatine becomes liquid and very frequently fine hair-like extensions ramify from the needle's path in the depth into the adjacent gelatine.

Agar-agar.—Forms a dry, grey white expansion.

Potatoes.—Produces an abundant although restricted dry white growth.

Blood Serum.—Liquefies the serum.

Remarks.—No sulphuretted hydrogen is produced in broth-cultures (Stagnitta-Balistreri). It is pathogenic to numerous animals, and produces wool-sorters' disease or malignant pustule in man. White mice die in twenty-four hours after inoculation. Chamberland and Roux (*Comptes rendus*, 1883, p. 1090) succeeded in producing a race of bacilli from anthrax blood permanently incapable of producing spores (asporogène anthrax), the virulence of which is not affected. For this purpose an addition of $\frac{1}{2000}$ to $\frac{1}{3000}$ of potassium dichromate is made to ordinary broth. Pasteur obtained anthrax vaccine by cultivating the bacilli in broth at between 42° and 43° C. Toussaint prepared vaccine by heating virulent anthrax bacilli for ten minutes at 55° C. It will not grow below 12–14° C., nor above 45° C. *B. subtilis* (see p. 417) resembles the anthrax bacillus, but has rounded ends and is not pathogenic to animals. For the effect of insolation on the bacillus see Chapter IX. Schild (*Zeitschrift f. Hygiene*, vol. xvi., 1894, p. 383) states that anthrax spores are destroyed in a formalin solution of 1 : 1000 in 1 hour (see note p. 285).

BACILLUS SUBTILIS (Hay bacillus)

LIQUEFIES GELATINE

Authority.—Ehrenberg.

Where Found.—In hay infusions, air, water, fæces, and in putrid liquids.

Microscopic Appearance.—Bacillus resembling the anthrax bacillus, but somewhat narrower and with rounded ends. It is about 6 μ long and three times as long as broad; grows out into long threads. The bacilli have flagella. It is motile, its movements being of a wobbling character. Forms egg-shaped shining spores about 1·2 μ long and 0·6 μ broad. They will bear exposure to dry heat of 120° C. for over one hour. (See Plate I. 2E, 2D.)

Cultures.—

Gelatine Plates.—The colonies become visible to the naked eye in about two days' time as small white dots in the depth, whilst on the surface they exhibit a very small liquefied circle of a greyish hue. Under a low power the depth colonies are irregular in contour with short spinose extensions in parts of the circumference, whilst the interior has a wavy structure, as if composed of coiled threads. With increasing size the internal structure becomes less defined, whilst the edge becomes uniformly spinose. In about two days' time the surface of the gelatine exhibits in places small cloudy expansions, which under a low power are seen to consist of parallel bands of fine threads arranged in a much contorted pattern. This appears to be the form assumed by the colonies on first reaching the surface of the gelatine, for these colonies in a day or two produce a liquefied surface with the usual spinose margin (Percy and G. C. Frankland, *Phil. Trans.*, 1887). (See Plate I. 2A, 2B, 2C.)

Gelatine Tubes.—Forms a long, funnel-shaped liquefying channel, the lower part of which throws out feathery lateral extensions. The whole of the gelatine becomes fluid and a tough white pellicle forms on the surface, and a large quantity of flocculent matter collects at the bottom of the tube.

Agar-agar.—Forms rapidly a white opaque expansion which becomes dry and copiously wrinkled and puckered.

Broth.—Renders the liquid turbid; produces a white deposit, and a pellicle which gradually increases in thickness and tenacity.

Potatoes.—Forms a moist white cream-like expansion over the whole surface.

Blood Serum.—It liquefies the serum, forming a wrinkled pellicle on the surface.

Remarks.—It is not pathogenic. It is strictly aërobic.

BACILLUS VERMICULARIS

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, 'Ueber einige typische Mikroorganismen im Wasser und im Boden,' *Zeitschrift f. Hygiene*, vol. vi., 1889, p. 384.

Where Found.—In water from the River Lea near Chingford. An organism obtained from water and subsequently described as *B. vermiculosus* by Zimmermann (*loc. cit.*, 1890) is doubtless identical with this.

Microscopic Appearance.—Large bacillus with rounded ends, in length about 2 to 3 μ long and about 1 μ broad. Forms extensive vermiform threads. Produces oval spores about 1·5 μ long and 1 μ broad. It is not motile.

Cultures.—

Gelatine Plates.—The colonies in the depth are irregular in contour. This irregularity increases as the liquefaction commences, and the colony approaches the surface. The periphery is seen to consist of closely-packed wavy bands of bacilli, whilst the centre of the colony looks irregular and wrinkled.

Gelatine Tubes.—Forms a moist shining grey expansion, whilst in the depth the path of the needle is indicated by a slight sword-like growth. Slow liquefaction of the gelatine takes place.

Agar-agar.—Produces a smooth shining greyish expansion which only extends slowly.

Potatoes.—Produces a thick irregular flesh-coloured pigment.

Remarks.—Powerfully reduces the nitrate to nitrite. (See p. 27.)

BACILLUS MEGATERIUM

LIQUEFIES GELATINE

Authority.—De Bary, *Vergleichende Morphologie und Biologie der Pilze.*

Where Found.—Isolated originally from cooked cabbage-leaves. Found in water by Tils, *Zeitschrift für Hygiene*, vol. ix., 1890, p. 312.

Microscopic Appearance.—Large slightly curved bacillus with rounded ends, 2·5 μ broad and 8 to 9 μ long. Characteristic granulation of the contents of the cell is visible. It has a great tendency to produce involution forms. It is motile, the movements being creeping. Forms spores at the end of the rod.

Cultures.—

Gelatine Plates.—Small round liquefying centres.

Gelatine Tubes.—Grows principally on the surface and liquefies the gelatine.

Agar-agar.—Produces a whitish expansion, whilst the agar becomes dark coloured.

Potatoes.—Yellow white cheese-like growth, restricted to the point of inoculation.

BACILLUS RAMOSUS (Wurzel bacillus)

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, 'Ueber einige typische Mikroorganismen im Wasser und im Boden,' *Zeitschrift f. Hygiene*, vol. vi., 1889, p. 388. Doubtless identical with the *Wurzel bacillus* (Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 126), and the *Bacillus implexus* described by Zimmermann as occurring in the Chemnitz water.

Where Found.—Originally in soil. Found by various investigators in water, and by the authors frequently in the rivers Thames and Lea, but not in deep well-water from the chalk.

Microscopic Appearance.—Much resembles *B. subtilis*. The individual bacilli are about 7 μ long and 1·7 μ broad, the ends being distinctly rounded. It gives rise to long threads, also spores. Is capable of only slight oscillatory movement.

Cultures.—

Gelatine Plates.—The colonies are seen to consist of cloudy centres with tangled root-like branches extending in every direction. Later liquefaction of the gelatine takes place. (See Plate II. 1A.)

Gelatine Tubes.—On the second day already a slight depression is visible on the surface, whilst the path of the needle in the depth has a grey woolly appearance. The whole contents of the tube become subsequently impregnated with fluffy ramifications and the gelatine becomes fluid, whilst a tough pellicle forms on the surface.

Agar-agar.—Grows rapidly over the whole surface, whilst in the depth the characteristic 'branching' is again visible.

Potatoes.—Produces a dry and uniform expansion which is almost quite white.

Broth. - Forms a light flocculent deposit, and produces later a tough and wrinkled pellicle on the surface.

Remarks.—It powerfully reduces the nitrate to nitrite. (See p. 27.)

BACILLUS MYCOIDES

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 324.

Where Found.—Found very frequently in the superficial layers of arable and garden soil. Also in water by Zimmermann, *loc. cit.*, and by Roux, *loc. cit.*, and in hail by Foutin, *loc. cit.* This organism resembles in many particulars the *B. ramosus*, but evidence as to its actual identity with the latter is still wanting.

Microscopic Appearance.—Bacillus from 1·6 to 2·4 μ long, and about 0·9 μ broad. It generally occurs in long threads, but the bacillar divisions of which are easily seen in stained preparations. It forms very lustrous oval spores about 1·3 to 1·48 μ long and 0·74 to 0·9 μ broad. It is motile.

Cultures.—

Gelatine Plates.—Forms a white, cloudy patch, in which later fine white interwoven threads become visible, and the branching of which resembles a mould. As soon as these thread-like growths reach the surface they expand, and the gelatine is liquefied.

Gelatine Tubes.—Rapid liquefaction of the upper layers of the gelatine, whilst in the depth hair-like ramifications, much branched and interwoven, extend from the needle's path into the gelatine. Later liquefaction extends throughout the tube, and a pellicle forms on the surface.

Agar-agar.—Fine mould-like ramifications extend on both sides of the needle's path on the surface.

Potatoes.—Forms in twenty-four hours a greyish white, slimy expansion, which later covers the whole surface.

Remarks.—In Wiedemann's *Annalen der Physik u. Chemie*, No. 8, 1893, Marchal states that the *B. mycoides* exerts a double action in soil in the production of ammonia, liberating ammonia from nitrogenous organic matter, and also denitrifying nitrates. See also action of *B. ramosus* on nitrates.

PROTEUS VULGARIS

LIQUEFIES GELATINE

Authority.—Hauser, *Ueber Fäulnis-Bakterien*, Leipzig, 1885.

Where Found.—In putrefying animal substances. In water (Zimmermann). Found also in sewage by Roscoe and Lunt, ' Chemical Bacteriology of Sewage,' *Phil. Trans.*, vol. clxxxii., 1892, p. 644. Found also in urine in cases of cystitis by Krogius (Helsingfors, 1892). The *Proteus sulfureus* found in water by Holschewnikoff (*Fortschritte der Medicin*, vol. vii. p. 201) resembles the above, and is probably identical with it.

Microscopic Appearance.—Slightly bent bacilli, about 0·6 μ broad, and of very variable length up to 3·75 μ; also gives rise to snake-like threads, resembling sometimes woven plaits of hair. Involution forms frequently occur. It is very motile, and has long cilia. No spore formation has been observed.

Cultures.—

Gelatine Plates.—The colonies are yellowish brown, with a bristly edge; from this margin spring processes consisting of coils of parallel bacilli, which spread like tendrils into the surrounding gelatine, also giving rise to most curious figures consisting of closely packed rows of bacilli, commonly called ' wandering or swarming islets.' In the depth characteristically shaped zoogloœa forms are often met with. Rapid liquefaction ensues.

Gelatine Tubes.—Liquefies the gelatine rapidly all along the needle's path; later, when all the contents are fluid, a whitish grey cloud is visible on the surface, whilst an abundant deposit, consisting of thick, crumbly fragments, collects at the bottom.

Agar-agar.—Forms a thin, spreading, moist, shining, greyish white expansion.

Potatoes.—Forms a dirty, smeary growth.

Remarks.—Pathogenic to rabbits and guinea-pigs.

PROTEUS MIRABILIS

LIQUEFIES GELATINE

Authority.—Hauser, *Ueber Fäulnis-Bakterien*, Leipzig, 1885.

Where Found.—In putrefying animal substances. In water (Zimmermann).

Microscopic Appearance.—Bacilli about 0·6 μ broad and of variable length, being sometimes almost round, and sometimes 2·0 to 3·7 μ long. It gives rise to involution forms more readily than the *Proteus vulgaris*, exhibiting spherical or pyriform structures from 3·75 to 7·0 μ in diameter. It is motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms a circular white expansion, which, under a low power, appears brownish and finely granular; the periphery is wavy and lobular, from which extensions run out into the surrounding gelatine. There is less movement to be seen in these liquefying expansions than in those formed by the *P. vulgaris*, but they exhibit, like the latter, zoogloœa forms. Liquefaction is less rapid than in the case of *P. vulgaris*.

Gelatine Tubes.—Forms an expansion, surrounded by a liquid, circular zone, filled with moving bacilli and threads. At the end of forty-eight hours a thick, moist, and shining pellicle is formed, and in from two to three days the whole contents of the tube are fluid.

Remarks.—Pathogenic to rabbits and guinea-pigs. It is facultatively anaërobic. No liquefaction takes place in the absence of oxygen.

PROTEUS ZENKERI

LIQUEFIES GELATINE

Authority.—Hauser, *Ueber Fäulnis-Bakterien*, Leipzig, 1885.

Where Found.—In putrefying animal substances. Included by Roux (*loc. cit.*) and Lustig (*loc. cit.*) amongst organisms found in water.

Microscopic Appearance.—Bacilli about 0·4 μ broad, and on an average about 1·65 μ long; rounder as well as longer forms are also found. It is motile.

Cultures.—

Gelatine Plates.—Forms a thick, whitish grey expansion, which can easily be removed from the surface. It gives rise to no zoogloœa figures in the depth, which serves to distinguish it from *Proteus vulgaris* and *Proteus mirabilis*. The gelatine is very slowly and slightly liquefied.

Gelatine Tubes.—Forms an expansion, becoming thinner by regular steps towards the periphery, from the edge of which numerous small rods and threads extend in a meandering fashion.

Remarks.—It is facultatively anaërobic. Pathogenic to guinea-pigs and rabbits.

BACILLUS PROTEUS FLUORESCENS

LIQUEFIES GELATINE

Authority.—Jaeger, 'Die Aetiologie des infectiösen fieberhaften Icterus,' *Zeitschrift f. Hygiene*, vol. xii., 1892, p. 525.

Where Found.—Isolated from the kidney and spleen of fowls which had died of some epidemic disease; also obtained from a stream into which the dead carcases were thrown. Jaeger states that he believes it to be identical with an organism which he isolated in cases of infectious feverish icterus which occurred amongst people who had bathed in contaminated water.

Microscopic Appearance.—Very variable in form. Sometimes short, thick bacilli, with rounded ends, mostly in pairs, in which the division is more or less distinct, according to the intensity of the stain. Forms also short threads, which are sometimes wavy. The poles often stain more intensely than the middle, leaving a colourless void. It is difficult to stain well; carbol-fuchsin, warmed on the cover-glass, yields the best results. It is not coloured by Gram's method. Is provided with numerous cilia, and is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—In from twenty-four to thirty-six hours, or after four days, small colonies, resembling bright drops of water, are visible. Under a low power they are circular, light yellow, smooth-rimmed and slightly granular; later the contour becomes somewhat lobular. When liquefaction begins the gelatine sinks in the shape of a funnel, and the colony resembles that of an advanced anthrax growth. The surface colonies recall a typhoid growth, the centre being, however, thick, lumpy, and white; later they resemble a well-developed anthrax colony, forming also characteristic Proteus figures and the typical isolated 'islands' in the solid gelatine.

Gelatine Tubes.—Forms an expansion on the surface which sinks, and the gelatine is liquefied in the shape of an air-bubble exactly resembling the cultures of the Comma bacillus. The gelatine becomes green fluorescent. A pellicle forms on the surface, which is sometimes thick and at times delicate, and wrinkled at the edge.

Agar-agar.—At 37·5° C. it forms in twenty-four hours growths resembling clear drops of water, and in forty-eight hours there appears a thick, yellowish white expansion, which produces a green fluorescence, and forms a flocculent deposit in the condensed water.

Potatoes.—Forms a thick, smeary, pale yellow expansion, which later becomes dark brown, and the potato assumes a bluish grey colour.

Broth.—Renders it turbid, and forms a pellicle occasionally.

Remarks.—It is pathogenic to mice.

TUBERCLE BACILLUS

(*Bacillus tuberculosis*)

Authority.—Koch, 'Die Aetiologie der Tuberkulose,' *Mittheilungen a. d. kaiserlichen Gesundheitsamte*, vol. ii., 1884.

Where Found.—In all tuberculous diseases of man and animals. Found in ditch-water by Fernandez, 'Infeccion tuberculosa por el agua contaminada,' *Revista de Medicina e Cirurgia pratica*, 1890.

Microscopic Appearance.—Very slender bacilli from 1·6 to 3·5 μ long. When stained with methylene blue they look thinner than when gentian violet or fuchsin is used. It is not motile. Spore formation is doubtful. (Fraenkel, *Grundriss d. Bakterienkunde*, 3rd edition, 1890, p. 380. Günther, *Einführung in das Studium der Bakteriologie*, 3rd edition, 1893, p. 221.) Stains with difficulty; Ziehl's carbol-fuchsin solution (p. 46) or Ehrlich's aniline water fuchsin solution (p. 45) may be employed. An ordinary cover-glass preparation, fixed by heating in the usual manner, should be placed in a small watch-glass containing the fuchsin solution, which is heated over a Bunsen flame until bubbles appear; the cover-glass is then removed, and placed preparation-side upwards in alcohol containing 3 per cent. of hydrochloric acid for one minute; it is then thoroughly washed and stained with a few drops of an aqueous alcoholic methylene blue solution; it is then washed and dried and passed again three times through the flame in the usual manner (Günther, *loc. cit.*, p. 222). The bacilli are stained by Gram's method.

Cultures.—

Gelatine.—No growth.

Glycerine-agar.—Grows abundantly, producing compact irregular growths dirty white in colour and folded and wrinkled on the surface. (Nocard and Roux add 6 to 8 per cent. of glycerine to ordinary nutritive agar-agar.)

Blood Serum.—Forms a very thin grey white expansion. No liquefaction ensues. With an addition of glycerine to the serum the growth is very vigorous, and a thick projecting faint white, mammellated expansion is produced (Nocard and Roux, *Annales de l'Institut Pasteur*, vol. i., 1887, p. 22). (See p. 19.)

Potatoes.—At 37° C. in from 12 to 20 days smooth whitish colonies appear on the potato, but the latter must be rendered alkaline and preserved in air-tight tubes. (Pawlowski.)

Glycerine Broth.—In veal broth plus 5 per cent. of glycerine small flocculent particles collect at the bottom of the tube, which at the end of three weeks have become very numerous (Nocard and Roux). It will also grow in beef and other broth.

Remarks.—This bacillus will neither grow below 29° C. nor at 42° C. The most favourable temperature lies between 37° and 38° C. In artificial cultures it retains almost its full complement of virulence even at the end of nine years of uninterrupted tube cultivation (Koch, *Tenth International Medical Congress*, Berlin, 1890, vol. i. p. 39). It is pathogenic to guinea-pigs, field-mice, rabbits and cats, but not to dogs, rats and white mice. For the effect produced by insolation on this bacillus see p. 354.

BACILLUS OF TETANUS

(Bacillus tetani)

LIQUEFIES GELATINE

Authority.—Nicolaier, *Deutsche med. Wochenschrift*, 1884, No. 52. Kitasato, 'Ueber den Tetanusbacillus,' *Zeitschrift f. Hygiene*, vol. vii., 1889, p. 225. Kitt, 'Ueber Tetanusimpfungen bei Hausthieren,' *Centralblatt für Bakteriologie*, vol. vii., 1890, p. 297.

Where Found.—In soil, in air, in water, and in pus from tetanus wounds.

Microscopic Appearance.—Straight bacilli with rounded ends, somewhat longer but scarcely broader than the bacillus of mouse septicæmia. It occurs singly, also in long threads. Forms spores in about thirty hours at 37° C., which are round and broader than the bacillus and are situated at one end. At 20° to 25° C. spore formation does not take place under seven days. In moist surroundings the spores will resist one hour's exposure to 80° C., but are killed by five minutes' exposure to 100° C. in the steam steriliser. The spores when dried on silk threads or mixed with soil retain their vitality and virulence for several months. It is slightly motile, but the spore-carrying bacilli are motionless. It can be easily stained with all the usual aqueous solutions of aniline colours, and it is also stained by Gram's method.

Cultures.—

Gelatine Plates.—When grown in an atmosphere of hydrogen the colonies at first resemble those of the hay bacillus (see p. 417). The centre is dense, and the periphery consists of a circle composed of fine short hair-like extensions of equal length. Liquefaction proceeds very slowly. Older colonies appear to be composed entirely of numerous isolated radial extensions, recalling the appearance of a mould.

Gelatine Tubes.—It is strictly anaërobic. In the depth of the gelatine a cloudy growth appears from which radial extensions penetrate the adjacent gelatine in all directions. Slow liquefaction ensues and gas is liberated.

Agar-agar.—Grows rapidly when 1·5 to 2 per cent. of grape sugar is added to the agar.

Blood Serum.—At from 34° to 38° C. soft circular depressions appear in the serum, due to a transparent and almost invisible delicate and colourless growth. After being kept at 37° C. for from six to ten days the serum becomes horizontally cleft. (Kitt.)

Broth.—Renders it very turbid.

Remarks.—It grows better at 37° C. than at 18° to 20° C., and no growth takes place below 14° C. It is very important that all media used for its cultivation should be quite freshly prepared (see p. 12). All the cultures emit an offensive odour. The bacillus may be isolated from tetanus-pus by inoculating portions of the latter on to agar-agar, and subsequently keeping the cultures at 36° to 38° C. for forty-eight hours, and then exposing them to a temperature of 80° C. for from three-quarters to one hour in a water-bath. The tetanus spores are then usually found in a pure condition, and may be inoculated on to fresh material and kept in an atmosphere of hydrogen. It is pathogenic to mice, rats, guinea-pigs, and rabbits, whilst more recently Kitt has found it fatal to horses, sheep, and dogs. For the action of light on tetanus filtrates see p. 385.

BACILLUS PYOCYANEUS (Gessard)

LIQUEFIES GELATINE

Authority.—Gessard, *De la Pyocyanine et de son Microbe*, Thèse de Paris, 1882; also Charrin, *Communication faite à la Société anatomique*, December 1884; Ernst, 'Ueber einen neuen Bacillus des blauen Eiters,' *Zeitschrift f. Hygiene*, vol. ii., 1887, p. 369. This author has found a variety of the *B. pyocyaneus* previously described which produces a bluish green colour, rapidly liquefies the gelatine, and gives rise to very little fluorescence.

Where Found.—In green pus. Found by Tils (*loc. cit.*) in the Freiburg water.

Microscopic Appearance.—Small slender bacillus, about as long as the bacillus of mouse septicæmia, but slightly broader. It occurs singly, but also in irregular groups. It forms spores (Flügge). It is very motile, and has one cilium attached to each bacillus (Loeffler).

Cultures.—

Gelatine Plates.—Forms irregular flat expansions, in the vicinity of which the gelatine is rapidly liquefied and assumes a green fluorescence. The depth colonies are circular and the highly refracting rim is light and granular.

Gelatine Tubes.—Forms in twenty-four hours a funnel-shaped liquid depression, whilst in the superficial layers the gelatine is fluorescent, and later the whole contents of the tube become beautifully green fluorescent.

Agar-agar.—Forms a moist greenish white expansion, and the whole of the agar becomes green fluorescent.

Potatoes.—Produces a red brown expansion confined to the point of inoculation. When the potato is treated with ammonia the growth becomes green, and when treated with an acid red, in colour.

Remarks.—Injected into the peritoneum of guinea-pigs, the latter die (Koch). Injected intravenously into rabbits, they do not die. The bacillus appears to increase in virulence when continually inoculated from one animal into another. For the effect of insolation on this bacillus, see p. 353.

BACILLUS FLUORESCENS TENUIS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbes. des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1891.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Short thick bacillus about 0·8 μ broad, and 1·0 to 1·85 μ long, with rounded ends, except in very young rods, when only one end is rounded. It occurs in groups, and in older cultures the rods consist of four to six individual bacilli. It is decolourised by Gram's method, which distinguishes it from *B. fluorescens longus*. Is capable only of oscillatory and rotatory movements. The same uncertainty as regards spore formation as in the *B. f. longus*.

Cultures.—

Gelatine Plates.—Forms thin shining, not always circular expansions, with an irregular denticulated edge. The surrounding gelatine is coloured green for some distance beyond the limit of the colony.

Gelatine Tubes.—Forms a grey white expansion, which after four days reaches the wall of the tube. The needle's path in the depth is clearly defined, but no further growth makes its appearance. The gelatine is coloured green. When *streaked* on a sloped gelatine surface, it forms a most delicate leaf-like expansion. No liquefaction takes place.

Agar-agar.—Forms a grey, smooth, shining, but not abundant expansion. The agar becomes gradually green.

Potatoes.—At first restricted to the path of inoculation, and appears as a thin greyish yellow shining expansion, which later becomes red brown in colour.

BACILLUS FLUORESCENS LONGUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Bacillus of very variable dimensions, longer and shorter, straight and bent rods, all occurring together, as well as semicircular or wavy threads. The bacillus itself is about 0·83 μ broad; the length of the shortest individuals is from 1·45 to 1·65 μ, the longest forms reach 14 μ and more. Is coloured by Gram's method. The shorter bacilli are very motile, the longer threads are stationary. Light unstained spots observed in the rod, but it is doubtful if they are spores.

Cultures.—

Gelatine Plates.—The colonies in the depth are small greenish white dots, whilst on the surface they form almost circular smooth expansions with a mother-of-pearl iridescence. They extend rapidly (in three days they reach often 9 mm. across) and are yellowish green in colour, and look as if yellow white threads had been drawn through them. Under a low power the depth colonies are sharply defined and yellowish in colour, whilst the contents have a convoluted and banded appearance. Similar convolutions are visible in the surface colonies, but the bands are broader and resemble the convolutions in the intestine of a small animal. No liquefaction takes place.

Gelatine Tubes.—Forms a thin but afterwards thicker expansion, at first blue, but later of a blue green fluorescent colour.

Agar-agar.—The agar becomes greenish yellow, and only a moderately thick expansion is seen.

Potatoes.—Forms a moist, shining, thin expansion, yellowish in colour, which extends over nearly the whole surface.

BACILLUS FLUORESCENS NON-LIQUEFACIENS

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 145.

Where Found.—In water. This is doubtless the same organism which was subsequently isolated from water and described by Adametz as *Fluorescirendes, blaugrünes Bacterium* (*Mitth. d. österr. Versuchsstat.*, Heft 2, 1888).

Microscopic Appearance.—Short fine bacilli with rounded ends. It is not motile.

Cultures.—

Gelatine Plates.—The surface colonies resemble fern-leaves, with a mother-of-pearl opalescence extending to some distance. No liquefaction takes place.

Gelatine Tubes.— Grows very slightly along the needle's path in the depth, and produces on the surface a fluorescent shimmer.

Agar-agar.—Forms a surface expansion producing a green colour.

Potatoes.—Grows rapidly, forming a diffused brownish growth; the surface assumes a bluish grey colour.

Remarks.—It is strictly aërobic.

BACILLUS AQUATILIS FLUORESCENS

Authority.—Lustig, *Diagnostik der Bakterien des Wassers*, p. 64.

Where Found.—Eisenberg is stated by Lustig (*loc. cit.*) to have found it in water.

Microscopic Appearance.—Short thin bacillus with rounded ends. Non-motile.

Cultures.—

Gelatine Plates.—The superficial colonies resemble a fern-leaf and look like mother-of-pearl.

Gelatine Tubes.—Grows only slightly in the depth, but produces a fluorescent and shining expansion on the surface. It does not liquefy the gelatine.

Agar-agar.—Develops on the surface and exhibits a green colour.

Potatoes.—Rapidly produces an extensive grey mass.

Remarks.—It is aërobic. This bacillus appears to be identical with Eisenberg's *B. fluorescens non-liquefaciens*, see p. 425.

BACILLUS VIRIDIS PALLESCENS

Authority.—Frick, *Virchow's Archiv für path. Anatomie*, vol. cxvi. p. 292.

Where Found.—In Freiburg water by Tils (*loc. cit.*).

Microscopic Appearance.—Rather larger and more slender than the typhoid bacillus. Often forms long threads. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms small dark colonies in the depth. On the surface the colonies form quite flat expansions, not rising above the surface of the gelatine, with well-defined and irregular periphery. The contents are finely granular. At first the colour is grey, but after twenty-eight hours it becomes much paler and turns to pale bluish green. The colonies are often slightly iridescent. No liquefaction takes place.

Gelatine Tubes.—Grows principally on the surface and develops but very slightly in the depth. The gelatine exhibits a blue green fluorescence, diminishing from above downwards.

Agar-agar.—Similar to gelatine.

Potatoes.—Forms a nut-brown moist expansion, whilst in the vicinity the potato turns a dirty violet colour.

Broth.—Forms a slight pellicle, which sinks to the bottom on the tube being shaken. In the upper layers the liquid becomes slightly bluish green.

Remarks.—It is strictly aërobic.

BACILLUS FLUORESCENS LIQUEFACIENS (Flügge)

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 289; also described by Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 75; A. Eugen Fick, *Ueber Microorganismen im Conjunctival-Sack*, Wiesbaden, 1887, p. 39; Percy Frankland (*B. viscosus*), *Zeitschrift f. Hygiene*, vol. vi., 1889, p. 391; Petruschky, 'Bakterio-chemische Untersuchungen,' *Centralblatt f. Bakteriologie*, vol. vii., 1890, p. 3; Ward, *Proc. Roy. Soc.*, vol. liii., 1893, p. 288. Probably also identical with the *B. fluorescens nivalis* found in ice-water and described by Schmolck, *Centralblatt f. Bakteriologie*, vol. iv..p. 544.

Where Found.—This organism, or slight variations of it, has been perhaps more frequently found in water than any other form. Fick found it also in the conjunctival secretion of a patient from whom a cataract had been removed.

Microscopic Appearance.—Very short bacillus, about 1 to 1·5 μ long and 0·5 μ broad; occurs chiefly in pairs, and exhibits a constriction in the middle. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Small white dots in the depth; on the surface they expand, reaching sometimes 3 mm. across, and after forty-eight hours the gelatine becomes liquefied and a well-defined circular depression is formed. Under a low power the brown dotted centre is surrounded by a yellow granular zone, which becomes grey white towards the edge. The whole gelatine assumes gradually a green fluorescence.

Gelatine Tubes.—In the path of the needle there is a whitish growth, whilst a funnel-shaped depression containing liquid gelatine forms on the surface; the liquefaction gradually stretches across the tube, and finally the whole contents are fluid and of a fluorescent green colour, whilst a thick white deposit collects.

Potatoes.—Produces a brownish expansion.

Milk.—Precipitates the casein and completely peptonises it. Yields an acid reaction (Ward, *loc. cit.*).

Remarks.—It is not pathogenic (Ward, *loc. cit.*).

Note.—The characteristic viscid nature of its growth in broth, gelatine, and agar, which was so noticeable that the name *viscosus* was given to it, appears either to have escaped the notice of other observers or to have been absent in the cultures of the organisms described by them. (Percy Frankland.)

BACILLUS TERMO

LIQUEFIES GELATINE

Authority.—Macé, *Traité pratique de Bactériologie*, 1892.

Where Found.—According to Macé, there exists a true *Bacillus termo* in water, whilst the *Bacterium termo* so often referred to by earlier writers probably includes a considerable number of different varieties, such as *Bacillus fluorescens liquefaciens*, *Proteus vulgaris*, &c. (Roux, *loc. cit.*, p. 331.)

Microscopic Appearance.—Thick rods, about 1·4 μ long and 0·8 μ broad, usually in pairs, sometimes in chains. It is very active and is possessed of flagella.

Cultures.—

Gelatine Plates.—In eight hours a small whitish colony with a greyish periphery is visible, surrounded by a zone of liquid gelatine. At the end of three or four days the centre is opaque, surrounded by a liquid 2 to 4 mm. in diameter. The periphery is pale and transparent, very sinuous, and sometimes lobular, exhibiting at 20° C. movements of the liquid which appear to displace the lobular projections, causing the whole colony to produce the illusory impression of an amœba. Sometimes the gelatine assumes a greenish tint in the vicinity of the colony.

Gelatine Tubes.—Forms a cup-shaped depression which becomes elongated into a funnel. The liquefaction soon reaches the walls of the tube.

Broth.—Renders the liquid turbid, and forms a light and brittle pellicle and only a slight deposit.

Remarks.—It is strictly aërobic. Is a powerful agent in the decomposition of animal and vegetable matters.

BACILLUS OF MOUSE SEPTICÆMIA (Koch)

(*Bacillus muriseptieus*)

LIQUEFIES GELATINE

Authority.—Gaffky, 'Ueber die Aetiologie der Wundinfektionskrankheiten,' *Mitth. a. d. kaiserlichen Gesundheitsamte*, vol. i., 1881, p. 80; Loeffler, *loc. cit.*, p. 135.

Where Found.—In the river Panke; also by Rintaro Mori (*Zeitschrift f. Hygiene*, vol. iv., 1888) in drain-water.

Microscopic Appearance.—Very small bacillus, 0·8 to 1·0 μ long and 0·1 to 0·2 μ broad; occurs frequently in pairs. It is not motile. Forms spores. Is stained by Gram's method.

Cultures.—

Gelatine Plates.—Does not grow on the surface; forms ill-defined colonies resembling small whitish clouds. Above each colony a small shallow depression is formed in the gelatine.

Gelatine Tubes.—Grows slowly, producing a delicate white diffused cloudy appearance. In strongly alkaline gelatine liquefaction sometimes takes place.

Agar-Agar.—Forms restricted yellowish white colonies.

Potatoes.—No growth.

Remarks.—Pathogenic to house mice; the latter, on being subcutaneously inoculated, die in from forty to sixty hours. Field-mice are immune.

BACILLUS OF RABBIT SEPTICÆMIA

(*Bacillus cuniculicida*)

Authority.—Koch-Gaffky, 'Experimentell erzeugte Septicämie,' *Mittheilung a. d. kais. Gesundheitsamte*, vol. i., 1881, p. 94.

Where Found.—In the river Panke, at Berlin. This bacillus is in all probability identical with the *Choléra des poules* of Pasteur and the *Bacillus der Vogel-septikämie* of Gamaleia (Eisenberg).

Microscopic Appearance.—A short bacillus about 1·4 μ long and 0·6 to 0·7 μ broad, rounded at the ends; several frequently join together and appear like threads, sometimes forming the figure 8. It is not motile. No spore formation observed. The poles stain more strongly than the centre, giving the appearance of a diplococcus. They are stained by Gram's method.

Cultures.—

Gelatine Plates.—Forms on the surface small round finely granular centres having an irregular edge. No liquefaction takes place.

Gelatine Tubes.—Produces a delicate and whitish serrated expansion.

Agar-agar.—Forms a whitish, shining, and fairly abundant expansion.

Blood Serum.—As on agar-agar.

Potatoes.—It will not grow at the ordinary temperature, but at 37° C. after some days a scanty yellowish grey expansion appears.

Broth.—Grows very slowly.

Remarks.—It is pathogenic for rabbits, mice, and birds. Roncali ('Dell' Azione del Veleno del Bacillus Tetani,' *Istituto d' Igiene sperimentale della R. Università di Roma*, vol. iii., 1893, p. 137) states that the *B. cuniculicida* may be rendered pathogenic to guinea-pigs when cultivated on agar containing the soluble products of the tetanus bacillus, these animals dying with septicæmia symptoms.

BACILLUS BREVIS (Kurzer Canalbacillus)

Authority.—Rintaro Mori, 'Ueber pathogene Bacterien im Canalwasser,' *Zeitschrift für Hygiene*, vol. iv., 1888, p. 53.

Where Found.—Found constantly in Berlin drain-water.

Microscopic Appearance.—Short bacillus with rounded ends about 2·5 μ long and 0·8 to 1·0 μ broad. The poles stain more intensely than the rest of the bacillus. It is not motile. Is not coloured by Gram's method.

Cultures.—

Gelatine Plates.—Grows very slowly at room-temperature; even after two to three weeks only pale yellow compact and almost microscopic centres are visible. No liquefaction takes place.

Gelatine Tubes.—After three weeks a thin yellowish expansion appears on the surface, whilst in the depth small centres are visible.

Agar-agar.—Grows at 35° C., and already in two to three days a yellowish growth is seen.

Blood Serum.—At 35° C. in two to three days produces a light grey expansion. The cultures in this and agar-agar only retain their vitality for from forty to fifty days.

Potatoes.—No growth.

Broth.—Forms a white cloudy deposit.

Remarks.—When subcutaneously inoculated into mice, the latter die in from sixteen to thirty hours. It is also pathogenic to guinea-pigs and rabbits.

BACILLUS CAPSULATUS

Authority.—Rintaro Mori, 'Ueber pathogene Bacterien im Canalwasser,' *Zeitschrift für Hygiene*, vol. iv., 1888, p. 52.

Where Found.—In Berlin drain-water. Resembles the *B. pneumoniae* of Friedländer.

Microscopic Appearance.—Bacillus exhibiting elliptical and rod-like forms 0·9 to 1·6 μ in size. Occurs very frequently, and in the bodies of animals exclusively, surrounded by a capsule about 4·5 μ long and 2·5 μ broad. Sometimes two are found joined end to end enclosed by one capsule. It is not motile. It is not stained by Gram's method.

Cultures.—

Gelatine Plates.—At room-temperature in twenty-four hours it forms porcelain-white shining pin-head colonies.

Gelatine Tubes.—It grows freely in the depth the whole length of the needle's path, forming on the surface a growth resembling the head of a nail. No liquefaction takes place.

Agar-agar.—Grows as on gelatine, but more favourably at a higher temperature of from 36° to 37° C. The growth is stringy.

Broth.—Produces a white turbidity, and after from three to four days a white pellicle forms on the surface, and more especially at the sides of the tube.

Potatoes.—Grows luxuriantly at 37° C., forming a yellowish moist stringy growth with slightly irregular edge. An abundant quantity of gas bubbles is produced.

Remarks.—Is pathogenic to mice, and, when injected into the pleural cavity, to rabbits.

BACILLUS HYDROPHILUS FUSCUS

LIQUEFIES GELATINE

Authority.—Sanarelli, 'Ueber einen neuen Mikroorganismus des Wassers,' *Centralblatt f. Bakteriologie*, vol. ix., 1891, pp. 193 and 222.

Where Found.—In well water supplied to the University of Siena.

Microscopic Appearance.—When taken from gelatine cultures the bacilli vary in size from 2 to 3 μ up to from 12 to 20 μ long, forming wavy threads, whilst some are so short that they have an egg-shaped or even ball-like appearance. Differences are also observable in their width. On agar-agar they are much more constant in form and mostly from 1 to 3 μ long, the smaller individuals are egg-shaped. No spore formation observed. It is very motile.

Cultures.—

Gelatine Plates.—Visible in from eighteen to twenty-four hours as circular and regular colonies with a smooth surface. They are greyish white in colour, but in direct light a faint bluish refraction is visible in their vicinity. The gelatine is so rapidly liquefied that their further progress cannot be watched.

Gelatine Tubes.—After twelve hours the gelatine is liquefied along the whole extent of the needle's path; the contents of the canal are turbid and contain white flocculent material. In three to four days the whole of the gelatine is fluid, and a thick whitish flocculent deposit is visible.

Glycerine-agar.—At 37° C., a few hours after inoculation a light blue diffused fluorescence is visible on the surface, after which an abundant and extensive growth appears and the condensed water becomes turbid. In from twenty-four to thirty-six hours large bubbles of gas appear in the depth. Later on the blue fluorescence disappears and the growth becomes thicker, and from a dirty grey it becomes brownish in colour.

Blood Serum.—The serum is rapidly liquefied all along the needle's path, already after twelve hours a fairly deep furrow being visible.

Potatoes.—After twelve hours along the needle's path a fine straw yellow pellicle is formed, which gradually becomes yellow, and in four to five days has turned so brown that it resembles exactly the growth of the Glanders bacillus on this medium.

Broth.—The liquid is turbid in twelve hours, and later a thin pellicle forms on the surface.

Remarks.—Pathogenic to frogs, toads, salamanders, fresh-water eels; also to warm-blooded animals, such as white mice, bats, guinea-pigs, newly-born kittens, dogs, and rabbits; also pigeons and fowls.

BACILLUS FUSCUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In water. Found also in water by Migula (*Zeitschrift f. Hygiene*, vol. viii., 1890, p. 357).

Microscopic Appearance.—Very variable in size; longer or shorter, straight and distinctly bent rods, with rounded ends and irregular contour; about 0·63 μ broad. It is not motile. No spore formation observed.

Cultures.—

Gelatine Plates.—In the depth small yellow brown dots, but on the surface raised pin-heads irregular and uneven. Under a low power the depth colonies are sharply defined and more or less circular, but frequently with an irregular and indented edge, resembling granular discs, and greyish or brownish yellow in colour. The surface colonies are brownish yellow in the centre, and are surrounded by a highly refracting shining border. The gelatine is not liquefied.

Gelatine Tubes.—Forms projecting button-growth, which later expands and becomes of a chrome-yellow colour.

Agar-agar.—Similar as in gelatine tubes.

Potatoes.—Forms a dark chrome-yellow crumbling expansion.

BACILLUS FUSCUS LIMBATUS

Authority.—Scheibenzuber, *Allgemeine Wiener med. Zeitung*, 1889, No. 16, p. 171.

Where Found.—In bad eggs. Tataroff (*loc. cit.*) describes a bacillus obtained by him from water (Harzfarbener bacillus) which he regards as identical with the above.

Microscopic Appearance.—Short bacilli, rarely occurring in threads. Very motile.

Cultures.—

Gelatine Plates.—Forms small brownish lumps with rounded edge; some are surrounded by a lighter zone two to three times as broad as the original colony. No liquefaction takes place.

Gelatine Tubes.—Does not expand much on the surface, but in the depth the needle's path is marked by short budding branches; the gelatine in the vicinity becomes brown, assuming the shape of a sack, the greatest width of which is directed upwards.

Agar-agar.—Forms a superficial expansion, the agar becoming more or less brown in colour.

BACILLUS BRUNNEUS

Authority.—Adametz and Wichmann, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888. Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 142.

Where Found.—Water.

Microscopic Appearance.—Fine slender bacillus. Not motile. Forms spores.

Cultures.—

Gelatine Plates.—Forms thick dirty white drop-like centres; later, after from ten to fourteen days, they become grey, and a brown pigment is produced in the under-part of the colony. No liquefaction takes place.

Gelatine Tubes.—It grows principally along the needle track in the depth; later a milk-white and slimy growth appears at the point of inoculation nearly 1 mm. thick. This mass becomes gradually grey, and the characteristic brown pigment separates out in the lower layers, whilst all along the needle track the colonies have a distinct brown colour.

Agar-agar.—Grows as in gelatine.

BACILLUS SAPROGENES II

Authority.—Rosenbach, *Mikroorganismen bei Wundinfektionskrankheiten*, Wiesbaden, 1884.

Where Found.—In perspiration from feet. Found by Tils on one occasion in large numbers in the Freiburg water (*loc. cit.*).

Microscopic Appearance.—Small slender bacillus.

Cultures.—

Gelatine Plates.—The depth colonies are yellow, and appear to be arranged near one another like balls. On the surface tough, slimy, smooth-rimmed and radially streaked colonies appear. The growth is so tough and compressed that the whole colony can be removed all at once with the needle (Tils, *loc. cit.*).

Agar-agar.—Forms in about twenty-four hours an expansion appearing to consist of numbers of individual tiny drops, later producing a clear tough slimy expansion. In the presence of air a most offensive odour is perceptible, but it is less intense in the absence of air.

Remarks.—When inoculated into the knee and pleural cavities of rabbits, these animals die, exhibiting purulent inflammation.

BACILLUS CLOACAE

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, p. 821.

Where Found.—Found in the Lawrence sewage, and described as one of the most common bacteria in this sewage.

Microscopic Appearance.—Short, plump oval bacilli, with rounded ends, about ·8 μ to 1·9 μ long, and ·7 μ to 1 μ broad. Variable in size, slightly longer and thicker on potato cultures than on agar. Occurs frequently in pairs. No spore formation observed. It is very motile.

Cultures.—

Gelatine Plates.—Is visible in from twenty-four to forty-eight hours as a round yellowish centre; on reaching the surface it forms a slight bluish expansion, with irregularly notched edges, and the gelatine is almost immediately liquefied. Under a low power the centre is dark, an outer translucent zone enclosed by a darker edge; the interior is finely granular. In from three to four days the whole plate is liquefied.

Gelatine Tubes.—Grows rapidly, and liquefies the gelatine all along the needle's path. Forms an iridescent scum on the surface, and a heavy, flocculent, whitish deposit. Will grow equally well in slightly acid gelatine.

Agar-agar.—Forms a moist, slimy porcelain-white surface growth. Grows also abundantly in the depth.

Milk.—Coagulates milk in about four days, rendering it strongly acid.

Broth.—Renders it turbid in two days. A white deposit is formed, and a light skin forms on the surface, which sinks on shaking the tube.

Potatoes.—Produces in two days an abundant raised yellowish white growth.

Remarks.—Reduces nitrates in bouillon. The following is the *analytical* composition of the solution employed:—

Albuminoid ammonia (from Merck's peptone) . .	8·88 parts per 100,000
Free ammonia	·92 „ „ „
Potassium nitrate	3·5 „ „ „

Unfortunately the synthetical composition of the medium is not stated.

BACILLUS UBIQUITUS

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, 'Purification of Sewage and Water,' p. 830.

Where Found.—Isolated from the Lawrence sewage; also found in natural waters and occasionally in the air. Jordan (*loc. cit.*) states that this bacillus resembles very closely the *B. candicans* isolated from soil and described by Percy and G. C. Frankland (*loc. cit.*), but differs chiefly in its power of reducing nitrates, whereas the *B. candicans* does not.

Microscopic Appearance.—Small short plump bacilli, closely resembling micrococci, 1·1 to 2 μ long and about 1 μ broad. Very variable in form. In broth cultures it exhibits a slight tendency to form short threads. It is not motile.

Cultures.—

Gelatine Plates.—Forms small roundish, often oval, colonies of a yellowish tinge. In two days the surface colonies resemble drops of milk, being white glistening projections. These centres slowly spread, become somewhat irregular, and take on a dull brownish cast. Under a low power the young colonies are smooth-rimmed, with a finely granular interior. No liquefaction takes place.

Gelatine Tubes.—Produces a nail-like growth, the colour being at first a lustrous porcelain white, but later a dull brownish grey.

Agar-agar.—Forms a good, whitish grey growth on the surface and along the inoculation line. The surface growth has a slightly metallic lustre.

Potatoes.—Exhibits a white and restricted shining growth.

Milk.—The milk is coagulated in eighteen hours at 37° C., and gives a strong acid reaction.

Broth.—Renders it turbid, forming a considerable flocculent deposit. On old cultures a thin skin forms on the surface, but this falls to the bottom on slightly jarring the tube.

Remarks.—Reduces nitrates vigorously. (See p. 433.)

BACILLUS SUPERFICIALIS

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, 'Purification of Sewage and Water,' p. 833.

Where Found.—Found frequently in Lawrence sewage.

Microscopic Appearance.—Fair-sized plump bacilli, about 2·2 μ long and 1 μ broad, with rounded ends. Generally occurs singly or in pairs. No spore formation observed. It is motile.

Cultures.—

Gelatine Plates.—Under a low power the colony is nearly round, but is divided by irregular lines into angular lumps, giving a somewhat cracked appearance to the whole colony. The surface colonies are round, homogeneous, finely granular expansions. To the naked eye the colony resembles a projecting translucent drop. The gelatine is slowly liquefied, after which the colony has a yellowish brown opaque centre, and a translucent edge.

Gelatine Tubes.—Grows slowly, requiring nearly ten days to liquefy the gelatine to the walls of the test-tubes. Grows almost solely on the surface, only the scantiest growth appearing along the inoculation line.

Agar-agar.—Forms a moist, lustrous, grey translucent growth. After several weeks the growth is still smooth and shiny, and has assumed a light brown tint.

Potatoes. - Refuses to grow on this medium.

Milk.—No visible change takes place, although the reaction is slightly acid.

Broth.—Renders the liquid turbid very slowly. No pellicle is formed, but a slight white precipitate is produced after some time.

Remarks.—It grows better at 37° C. than at 21° C. No reduction of the nitrates observed. (See p. 433.)

BACILLUS RETICULARIS

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, 'Purification of Sewage and Water,' p. 834.

Where Found.—In effluent from Lawrence sewage.

Microscopic Appearance.—Long, rather slender bacillus, about 5 μ long and 1 μ broad, with slightly rounded ends; often occur in strings of eight to ten loosely connected individuals. No true spore formation observed. Exhibits a slow sinuous motion.

Cultures.—

Gelatine Plates.—In the depth the young colonies send out long spiral filaments, which give a hazy appearance to the colony. Under a low power these radiating centres resemble jelly-fish with streaming tentacles. The surface colonies form a considerable and irregular expansion, and the gelatine is slowly liquefied. This takes place so slowly that the liquid evaporates almost as soon as formed, and the colonies then resemble slight hollows or cups in the gelatine. The surface of these cups presents a mottled appearance, as if it were covered with fine irregular network or reticulations.

Gelatine Tubes.—In two days the upper part of the growth has the appearance of a cup with 'flaring' edges. The gelatine, as in the case of the plates, is liquefied very slowly, and the cup has the same reticulated structure described above. In three days the filaments begin to shoot out from along the inoculation line, but they do not often reach any very great length.

Agar-agar.—Dull dry raised growth, and but a feeble development in the depth.

Potatoes.—Forms a white, dull and dry growth, which later assumes a characteristic woolly appearance.

Broth.—The liquid becomes slowly turbid, and a slight stringy precipitate forms.

Milk.—It is slowly coagulated, and gives an acid reaction.

Remarks.—Rapidly reduces nitrate to nitrite. (See *B. cloacae*, p. 433.) The bacillus grows much better at 37° than at 21° to 23° C.

BACILLUS CIRCULANS

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, p. 831.

Where Found.—Found occasionally in the Lawrence tap-water (from the Merrimac River).

Microscopic Appearance.—Long slender bacilli about 2 to 5 μ long and 1 μ broad, with rounded ends; generally single, but sometimes hanging together in twos and fours. Forms small oval spores in from three to four days in the end of the rod. It is very motile.

Cultures.—

Gelatine Plates.—Round brownish colonies are visible in two days. The gelatine is liquefied, and under a low power the rapid motion of the individual bacteria gives to the whole fluid mass the appearance of being in circulation. In three days the movement usually ceases; the interior looks coarsely granular with flocks of bacteria scattered irregularly here and there. The colonies form a round, deep, even depression, which spreads very slowly; the contour sometimes becomes somewhat lobed instead of being smooth and even.

Gelatine Tubes.—Grows slowly, forming a liquefying cone-like depression, at the bottom of which a deposit collects. It grows well in a slightly acid medium.

Agar-agar.—Forms a very thin translucent surface growth; grows also in the depth.

Potatoes.—Grows slowly and scantily, hardly distinguished in colour from the potato itself.

Milk.—Slowly coagulates the milk with a slight acid reaction. When cultivated for several months in artificial media it no longer is able to coagulate milk.

Broth.—Renders it turbid in from three to four days, forming a considerable slimy deposit. No pellicle is formed.

Remarks.—Slowly reduces nitrates to nitrites. (See under *B. cloacae.*)

BACILLUS HYALINUS

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, 'Purification of Water and Sewage,' p. 835.

Where Found.—Isolated from the sand of a tank used for filtering sewage at Lawrence.

Microscopic Appearance.—Large, long, stout bacilli, with rounded ends. Usually occurring in short strings. No spore formation observed. About 3·6 to 4 μ long and 1·5 μ broad. Very motile.

Cultures.—

Gelatine Plates.—Grows rapidly and is visible in twenty-four hours. The centre is dark, surrounded by a broad, translucent zone, which gives a hazy appearance to the colonies. Under a low power the contents are coarsely fibrillar, with short fibrils radiating from the edge. The colonies in two days are about 1 and $1\frac{1}{2}$ c.c. in diameter, the contour is evenly round, the interior slightly translucent; the rim is distinct, opaque, yellowish, whilst towards the edge the radiating fibrils are still visible.

Gelatine Tubes.—Forms in two days a long, narrow, funnel-shaped growth. The gelatine, which is rapidly liquefied, is at first cloudy, and a precipitate forms at the bottom of the funnel. In about eight days a lustrous, tenacious, white scum forms on the surface; the gelatine, which is completely fluid, is perfectly transparent, and at the bottom of the tube there rests a slight flocculent deposit.

Agar-agar.—Forms rapidly a spreading, dry, dull greyish growth. It is tough and rather thin. After about four or five days small warty projections appear. It grows well in the depth.

Potatoes.—Forms a spreading, dry, whitish grey growth, but less rapidly than on agar. Later small protuberances appear on the surface.

Broth.—Renders it turbid, with a thick pellicle and a stringy precipitate.

Milk.—In seven days strongly coagulated. Acid reaction.

Remarks.—It grows better at 37° than at 21° or 23° C. Reduces nitrates vigorously and rapidly. (See under *B. cloacae*, p. 433.)

BACILLUS DELICATULUS

LIQUEFIES GELATINE

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, p. 837.

Where Found.—In effluent from Lawrence sewage.

Microscopic Appearance.—Medium-sized, plump bacilli, 2 μ long and 1 μ broad, often joined in pairs and in short strings. Very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—When young the colonies are whitish, homogeneous, with a regular, radiating edge. In two days, at the temperature of the room, the gelatine becomes liquefied; later the centre becomes darker than the surrounding zone.

Gelatine Tubes.—In two days the gelatine is liquefied the length of the needle's path, and in about seven days the whole contents of the tube are fluid and cloudy. A thick, whitish skin forms on the surface, and a heavy, flocculent, brownish precipitate at the bottom. Grows nearly as well in slightly acid gelatine as in alkaline.

Agar-agar.—Forms at first a crinkled greyish growth, which when older becomes porcelain-white and glistening. Grows well both on the surface and below.

Potatoes.—A grey, spreading and flat growth.

Milk.—The milk is coagulated, and gives a strong acid reaction.

Broth.—The liquid quickly becomes turbid, and a white precipitate and scum are produced.

Remarks.—Reduces nitrate to nitrite very rapidly and completely (see *B. cloacae*). It is very sensitive to low temperatures. At about 15° C. it refuses to grow at all. After several weeks no living bacilli were obtained from tube cultures.

BACILLUS RUBESCENS

Authority.—Jordan, *A Report on certain Species of Bacteria observed in Sewage*, State Board of Health, Massachusetts, 1890, 'Sewage and Water,' p. 835.

Where Found.—In the Lawrence sewage.

Microscopic Appearance.—Large, long bacilli, about 4 μ long and 9 μ broad, with well rounded ends. Often occurs in pairs and short strings. Many of the individual bacilli are slightly curved. No spore formation observed. Slow and sluggish movement.

Cultures.—

Gelatine Plates.—Grows slowly. The depth colonies are usually round, sometimes oval. They form pin-head, porcelain-white, drop-like projections on the surface. Later they become slightly brownish.

Gelatine Tubes.—Grows slowly and chiefly on the surface, forming a porcelain-white, nail-head projection. Grows only slightly in the depth. No liquefaction ensues.

Agar-agar.—Grows rapidly, producing a white lustrous and smooth expansion. Later this becomes crinkly, and the whole surface is much wrinkled. In three weeks old cultures a slight pinkish tinge can be seen.

Potatoes.—Produces a luxuriant, light-brown, raised growth, which later becomes pink and flesh-pink in colour. The potato itself is not coloured.

Milk.—The milk is not coagulated, and in long-standing cultures a slight pinkish tinge is observed at the surface.

Broth.—Forms a heavy white precipitate, and the liquid is rendered turbid. A thick, tenacious skin forms much later on the surface, and the main body of the liquid is then clear.

Remarks.—No change in the nitrates after fifty days. (See under *B. cloacae*, p. 433.)

BACILLUS ERYTHROSPORUS

Authority.—Eidam, *Cohn's Beiträge zur Biologie der Pflanzen*, vol. iii. p. 128. Flügge, *Die Mikroorganismen*, 1886, p. 288.

Where Found.—Found originally in putrefying liquids by Eidam, later by Cohn and Miflet; more recently in drinking-water by Bolton (*Zeitschrift f. Hygiene*, vol. i., 1886) and by Migula (*Zeitschrift f. Hygiene*, vol. viii., 1890, p. 357).

Microscopic Appearance.—Slender bacillus with rounded ends; forms also short threads. It is very motile. From two to eight oval-shaped spores appear at the ordinary temperature in each rod; they sometimes extend beyond the wall of the bacillus, and are characterised further by their distinctly dirty red colour. Even when the bacillus is stained with methylene blue, the spores retain their reddish colour.

Cultures.—

Gelatine Plates.—Forms white centres, which under a low power are seen to have a sharply-defined but irregular edge. The brown central portion is surrounded by a brighter greenish yellow zone. On the surface, after the colony has expanded, distinct wavy lines are seen to radiate from the centre to the periphery, which becomes much lobulated and serrated. A green fluorescence surrounds each colony. The gelatine is not liquefied.

Gelatine Tubes.—Grows abundantly, both on the surface and along the needle's path in the depth, and the whole tube gradually assumes a green fluorescent colour.

Potatoes.—Produces a restricted growth, at first reddish but later nut-brown in colour.

BACILLUS LATERICEUS ('Ziegelrother Bacillus')

Authority.—Adametz and Wichmann, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—A bacillus three to five times as long as broad, forming short, often bent threads. It is not motile.

Cultures.—

Gelatine Plates.—Small dot-shaped sealing-wax-red colonies, which under a low power appear circular, finely granular, and of a brown-red colour. The centre is dark and is surrounded by a lighter peripheral zone. No liquefaction takes place.

Gelatine Tubes.—Forms a raised, slimy and sealing-wax-red expansion, and grows but slightly in the depth.

Potatoes.—Expansion of sealing-wax-red colour.

BACILLUS RUBIDUS

LIQUEFIES GELATINE

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 88.
Where Found.—In water.
Microscopic Appearance.—Medium-sized bacilli with rounded ends, often forming long threads. It is very motile.
Cultures.—
Gelatine Plates.—Round finely granular liquefying colonies, with a smooth rim and coloured centre.
Gelatine Tubes.—Liquefies the gelatine, slowly producing a brownish red pigment.
Agar-agar.—Forms a rapidly spreading brownish red expansion.
Potatoes.—Produces a fine brownish red-coloured growth, which is not restricted to the point of inoculation.
Blood Serum.—Liquefies the serum, producing a red pigment.

Remarks.—It will not grow at higher temperatures.

BACILLUS PRODIGIOSUS

LIQUEFIES GELATINE

Authority.—Ehrenberg. See also 'Ueber einige durch Bakterien gebildete Pigmente,' Schroeter, *Beiträge zur Biologie der Pflanzen*, Heft ii., 2nd edition, Breslau, 1881, p. 109. See also, 'Studies on some new Microorganisms obtained from Air,' Percy and G. C. Frankland, *Phil. Trans.*, vol. clxxviii., 1887, p. 284.
Where Found.—On moist bread, potatoes, etc. Found in water by Tils (*loc. cit.*) and Percy Frankland (*loc. cit.*).
Microscopic Appearance.—The cells are rather longer than broad, the largest forms being about 1·7 μ in length and about 1 μ in width; they are frequently found hanging together in pairs. It is not motile. (See Plate I. 1d.)
Cultures.—
Gelatine Plates.—After two days the colonies are visible to the naked eye as circular depressions, each having a red centre. Under a low power the less developed colonies in the depth are devoid of red colour, are finely granular, and have a very irregular contour. The surface colonies, on the other hand, have a distinctly red nucleus, surrounded by a very thin and finely granular brownish growth, having a very irregular contour. (Percy Frankland.) (See Plate I. 1b., 1c.)
Gelatine Tubes.—Grows very rapidly, liquefying the gelatine in the form of a conical sack, which soon extends across the tube at the top, and, gradually passing downwards, involves the whole tube. The liquid is very turbid, with an abundant flocculent deposit of an intensely crimson colour. Near the surface there is generally seen adhering to the glass a thin layer of still darker red-colouring matter, which has the peculiar fluorescence of an aniline colour when in a concentrated state. (Percy Frankland.) (See Plate I. 1a.)
Agar-agar.—Grows very rapidly, producing a deep, blood-red, smooth and shining expansion, the colour being restricted to the surface.
Blood Serum.—As on agar, only the serum becomes liquefied.
Potatoes.—Grows luxuriantly, producing a magnificent red pigment, which has a metallic brilliancy upon it.
Broth.—Renders the broth turbid, and produces at first a white deposit, which later becomes pinkish.

Remarks.—By long-continued culture it often loses its power of pigment-production, which may generally be restored by cultivation on potatoes.

BACILLUS RUBEFACIENS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Slender bacillus about 0·32 μ broad and 0·75 to 1·65 μ long, with rounded ends. Occurs in pairs or more, and is often found lying side by side. It is very motile.

Cultures.—

Gelatine Plates.—In the depth round or bean-shaped colonies are visible, whitish in colour, with a touch of yellow red. The surface colonies form flat expansions, greyish in colour, with a suggestion of red. Under a low power the depth colonies are circular, granular, smooth-rimmed, yellowish or brownish in colour. No liquefaction ensues.

Gelatine Tubes.—Forms a somewhat thick whitish grey expansion, which later becomes yellowish. The gelatine between the growth and the walls of the tube assumes a bluish white opalescence. Develops well in the depth. In old cultures the gelatine often becomes of a light wine-red colour. When *streaked* it forms a thin bluish grey shining expansion, with a delicately serrated edge; later it becomes somewhat folded.

Agar-agar.—Forms a smooth bluish grey raised expansion.

Potatoes.—Forms a fairly abundant greyish yellow, later brownish red, expansion, which on the second day renders the potato pinkish.

'DER ROTHE BACILLUS'

LIQUEFIES GELATINE

Authority.—Lustig, *Diagnostik der Bakterien des Wassers*, 1893, p. 72.

Where Found.—Found on one occasion in river-water.

Microscopic Appearance.—Small bacillus with rounded ends, generally two or three times as long as broad, but its dimensions and arrangement vary according to the temperature and material used for its cultivation. It is very motile; the filaments are also motile When the cells become more coloured its movements become oscillatory only. Forms spores.

Cultures.—

Gelatine Plates.—In forty-eight hours the surface colonies resemble grey dots with a red centre. Under a low power they are circular with serrated edge and granular surface, whilst the centre is a raspberry-red colour. The gelatine becomes liquefied, the colony sinks, and the red colour extends in all directions. In from four to six days the whole plate is fluid.

Gelatine Tubes.—At the end of twenty-four hours a small funnel-shaped depression is visible at the point of inoculation, in the centre of which the characteristic pigment is found. A thin transparent thread of dirty white liquid marks the needle's path in the depth. The liquefaction proceeds until finally the whole contents of the tube are fluid, consisting of a slimy glutinous material, raspberry-red in colour.

Agar-agar.—At the ordinary temperature a moist, crimson lake, and shining expansion appears, but at 37° to 40° C. only a milk-white growth is visible, which, even when kept for weeks, does not become red.

Potatoes.—Grows rapidly, producing a sticky, slimy, raspberry-red expansion which covers the whole surface, and later assumes a metallic colour.

Broth.—Renders it turbid and produces a red pigment at the ordinary temperature.

Sterilised Distilled Water.—It does not develop, and the water remains quite clear. On examining this water in suspended drops motionless shining bacillar forms with refracting protoplasm are visible. After thirty days this water, when inoculated into gelatine, gives rise to typical cultures of the bacillus.

BACILLUS RUBER ('Bacille rouge de Kiel')

LIQUEFIES GELATINE

Authority.—Breunig, *Bakteriologische Untersuchung des Trinkwassers der Stadt Kiel*, 1888; also Laurent, 'Etude sur la Variabilité du Bacille rouge de Kiel,' *Annales de l'Institut Pasteur*, vol. iv., 1890, p. 465.

Where Found.—In the water supply of Kiel.

Microscopic Appearance.—In a recent potato culture the bacillus varies in length from 2·5 to 5 μ and in breadth from 0·7 to 0·8 μ. In milk and broth the dimensions are similar, but in old potato cultures it may reach 8 and 10 μ in length. After being cultivated for four or five hours on potatoes at 35° C., the bacilli are slightly motile, the movement depending upon the presence of oxygen in the culture material, ceasing in its absence. (Laurent.)

Cultures.—

Gelatine Plates.—The colonies in the depth are pale yellow, oval, and with a regular or sinuous periphery. Seen with the naked eye they are white. The surface colonies are blood-red in colour and form extensive expansions with a sinuous periphery; they are surrounded by a clear zone, and after the fifth day the gelatine is slowly liquefied. The colonies do not become red, only those which are nearest the surface become slightly rose-coloured after six or seven days. (Laurent.)

Gelatine Tubes. - The gelatine is liquefied and becomes of a bright red colour. In the depth of the tube bubbles of gas are often produced.

Potatoes.—At 30° to 35° C. it grows rapidly, and in twenty-four hours the whole surface is covered with a purplish red growth.

Broth.—In neutral veal broth it renders the liquid very turbid in twenty-four hours, colouring it pale pink.

Milk.—At 35° C. the milk is coagulated in twenty-four hours, but no trace of colour is visible. At ordinary temperatures the coagulation takes place much more slowly, and the surface of the liquid becomes blood-red in colour, which gradually extends to the lower strata of the liquid.

Remarks.—It will hardly grow at all below 10° C., and will not develop at 42° C. The most favourable temperature is between 30° and 35° C. In acid media it grows extremely slowly, and no trace of colour is produced. It is very sensitive to insolation, and is destroyed when exposed to the direct solar rays for five hours. (Laurent.)

See p. 352.

BACILLUS CUTICULARIS ALBUS

Authority.—Tataroff, *Die Dorpater Wasserbakterien*, Dorpat, 1891, p. 24.

Where Found.—In Dorpat water, occurring occasionally.

Microscopic Appearance.—Somewhat thick double bacillus, with a constriction in the middle and rounded ends. About 3·2 μ long. Forms variously bent threads when grown on agar-agar. Most of the threads contain spores. It is very motile, but the agar forms exhibit only lively oscillatory movements.

Cultures.—

Gelatine Plates.—The surface colonies are irregular in shape and exhibit a blue white opalescence. Under a low power they are seen to be brownish in colour, with an irregular edge and granular contents. In the depth they are oval or circular, with a smooth rim. No liquefaction takes place.

Gelatine Tubes.—Produces an irregular white rosette-shaped shining expansion which in five days covers the surface of the gelatine. In the depth a shining white growth appears, which soon becomes granulated and shining. Later it grows out into the surrounding gelatine in the shape of large rounded lappets which reach to the walls of the tube.

Agar-agar, Glycerine-agar and Blood Serum.—Forms a luxuriant and shining white expansion and a white deposit.

Broth.—Renders it turbid, producing a white deposit, and flocculent particles float in the liquid. On the surface a whitish pellicle is formed.

Potatoes.—Grows as a brownish-coloured, thick, moist, shining, irregularly-raised expansion, which later becomes red brown and yellow brown in colour. It will not grow at a higher temperature on this medium.

'WEISSER BACILLUS' (Tataroff)

Authority.—Tataroff, *Die Dorpater Wasserbacterien*, Dorpat, 1891, p. 35.

Where Found.—In well-water from Dorpat. This bacillus differs from *Bacillus albus* inasmuch as it has rounded ends and is not motile. It resembles in nearly all respects (Tataroff, *loc. cit.*, p. 37) the *Bacillus candicans* obtained from soil and described by Percy and G. C. Frankland, *Zeitschrift für Hygiene*, vol. vi. p. 397.

Microscopic Appearance.—Slender double bacillus, nearly 1·5 μ long, with rounded ends. In stained preparations the small bacilli look short and oval. In broth threads are formed. It is not motile.

Cultures.—

Gelatine Plates.—White pin-head colonies, resembling bits of shining porcelain. Under a low power the depth colonies are either round or oval, faintly brown in colour, and with granular contents. The surface colonies are brown white with a touch of yellow, shining, circular, with sharp contour, although occasionally deep indentations are seen. No liquefaction takes place.

Gelatine Tubes.—Produces a shining somewhat thick porcelain-white expansion. Later the colour becomes grey white, and the gelatine is cleft by bubbles of gas. In the depth it produces a sword-like streak.

Agar-agar.—Shining, milk-white, moist and shiny expansion, and a white deposit collects in the condensed water.

Glycerine-agar.—Luxuriant and shining white growth covering the whole surface. Later it becomes greyish, and finally of a dirty white colour tinged with yellow.

Blood Serum.—White slimy and moist expansion.

Broth.—Renders it very turbid, producing a considerable amount of brownish white deposit.

Potatoes.—Forms a moist, shining, brown white expansion which does not extend very far from the point of inoculation.

BACILLUS ALBUS ('Weisser Bacillus')

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 171.

Where Found.—In water. This is doubtless the same organism as that found in water and described as 'Der weisse Bacillus' by Maschek (see p. 480).

Microscopic Appearance.—Short bacillus with blunted ends; often several are seen joining on end to end. It is motile.

Cultures.—

Gelatine Plates.—Round white pin-head colonies. No liquefaction of the gelatine takes place.

Gelatine Tubes.—Grows slowly, producing a whitish streak in the depth and forming a white pin-head on the surface as on the plates.

Agar-agar.—Produces a milk-white expansion.

Potatoes.—Forms a dirty yellow white growth restricted to the point of inoculation.

Remarks.—It will not grow at higher temperatures.

BACTERIUM LUTEUM (List)

Authority.—List, *Die Bakterien der Nutz- und Trinkwässer*, Adametz. Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Elliptical cells from 1·1 to 1·3 μ long. It is not motile.

Cultures.—

Gelatine Plates.—Forms irregular, slimy, orange yellow centres which gradually form flat expansions. Under a low power they appear to consist of club-shaped coarse granular zooglœa masses, each of which is made up of several pieces. No liquefaction takes place.

Gelatine Tubes.—Forms an orange yellow expansion on the surface, but grows slowly in the depth.

Milk.—Coagulates milk.

Remarks.—Grows at room temperature, but better at 30° C.

'GOLDEN YELLOW WATER BACILLUS'

Authority.—Adametz-Wichmann, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water. Also by Tils (*Zeitschrift f. Hygiene*, vol. ix., p. 307) and by Tataroff (*Die Dorpater Wasserbacterien*, Dorpat, 1891, p. 62).

Microscopic Appearance.—Bacilli, two or three times as long as broad. Moves slowly.

Cultures.—

Gelatine Plates.—The surface colonies are yellow shining dots, which only develop slowly. Under a low power the depth colonies are seen to be oval or circular, yellow and granular, whilst the surface colonies are round, with a smooth rim, and yellow in colour. No liquefaction of the gelatine takes place.

Gelatine Tubes.—The surface growth is red, but in the depth along the needle's track a shining yellow growth is seen.

BACILLUS HELVOLUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Short bacilli which occur at first mostly in pairs or in fours, but later form regular threads. They are about 0·5 μ broad and 1·5 to 2·5 μ and even 4·5 μ long. Exhibit only rotatory movements. Spore formation has not been determined with certainty.

Cultures.—

Gelatine Plates.—Forms in the depth small, circular, bright yellow dots, and on the surface a distinctly raised, drop-shaped colony of a pale yellow colour. The latter rests in a soft depression. Under a low power the depth colonies are pale yellow or pale brown, circular, granular and smooth-rimmed. The surface colonies are also for a time smooth-rimmed, but the periphery becomes gradually more irregular.

Gelatine Tubes.—Forms a button-like growth on the surface, of a full Naples yellow colour; it becomes larger until it almost reaches the wall of the tube, and the gelatine then assumes a saucer-like depression and becomes fluid. There is but little growth in the depth, and the liquefaction only proceeds slowly.

Agar-agar.—Abundant growth, of a full Naples yellow colour.

Potatoes.—Grows abundantly, forming a somewhat thick, shining, yellow expansion, which sometimes assumes a greenish tint.

BACILLUS OCHRACEUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply. This bacillus was isolated and described previously by Fazio, *I Microbi delle Acque minerali*, Napoli, 1888.

Microscopic Appearance.—Bacillus from 0·65 to 0·75 μ broad, and 1·25 to 4·5 μ long, with rounded ends. It occurs in pairs also, forming longer threads. In stained preparations it sometimes appears to be enveloped in a capsule. Slow snake-like movement.

Cultures.—

Gelatine Plates.—Forms in the depth small pale yellow ball-shaped colonies; the gelatine above becomes liquid and the colony rests finally in a cup-shaped depression. The colour becomes more intense, and is of a golden yellow ochre. Under a low power they appear as granular and brownish yellow discs, covered later with small wart-like protuberances.

Gelatine Tubes.—Forms a funnel-shaped depression, but when the liquefaction has extended right across the tube it proceeds more slowly. A pale yellow precipitate collects, which later becomes yellow ochre in colour, and when shaken renders the liquid cloudy and of a pale yellow ochre colour.

Agar-agar.—Forms a somewhat restricted yellow ochre expansion.

Potatoes.—Forms a thin yellow ochre expansion.

'LEMON-YELLOW BACILLUS'

LIQUEFIES GELATINE

Authority.—Maschek, *Die Bakterien der Nutz- und Trinkwässer*, Adametz, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Short bacillus. Lively pendular movements.

Cultures.—

Gelatine Plates.—Forms circular yellowish white colonies; the centre is light brown, with radial extensions towards the periphery.

Gelatine Tubes.—Forms a nail-head growth of a yellow colour. The gelatine is liquefied and assumes a lemon-yellow colour.

Agar-agar.—Forms a lemon-yellow surface expansion.

Potatoes.—Forms a lemon-yellow expansion.

'ORANGE-RED WATER BACILLUS'.

Authority.—Adametz-Wichmann, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Long, very thin bacillus. It is not motile.

Cultures.—

Gelatine Plates.—Grows very slowly, forming orange red centres. Under a low power they are finely granular, brown in colour, and either round or oval. The depth colonies appear to be colourless.

Gelatine Tubes.—Grows slowly on the surface, forming a slimy moist shining expansion of an orange red colour. No liquefaction takes place.

Remarks.—As far as the above description goes, it resembles the *B. aurantiacus*, p. 449, but the microscopic appearance does not correspond.

'YELLOW BACILLUS' (Lustig)

LIQUEFIES GELATINE

Authority.—Lustig, *Diagnostik der Bakterien des Wassers*, 1893, p. 78.

Where Found.—Frequently found in the Cagliari water supply.

Microscopic Appearance.—Short, thin bacillus with rounded ends, occurring usually in long, bent or spiral-shaped threads, consisting sometimes of 100 individual bacilli. It is not motile.

Cultures.—

Gelatine Plates.—After three days small grey dots appear, which under a low power are seen to be round, oval, or irregular in shape, golden yellow in colour, and with an irregular serrated edge. From the periphery delicate extensions stretch into the surrounding gelatine. The surface colonies are similar in appearance. Later on the gelatine in the immediate vicinity becomes liquid, and at the end of ten days the colony is seen to rest in a hollow, having lost its shape, but still of a golden yellow colour.

Gelatine Tubes.—Grows slowly, forming at the end of two days a small slimy drop resembling chloride of gold in colour. In the depth a yellow thread-like growth appears, from which fine, short, lateral extensions penetrate the gelatine. About the fourth day a small funnel-shaped depression is visible, at the bottom of which lies a slimy, golden yellow drop; later on the sides of the funnel also become coated with yellow slime. The liquefaction proceeds slowly.

Agar-agar.—Grows more rapidly than on gelatine, and produces a straw-yellow expansion.

Potatoes.—Forms a very restricted growth, coffee-yellow in colour.

Remarks.—It will only grow between 15° and 22° C.

BACILLUS FLAVOCORIACEUS

Authority.—Adametz and Wichmann, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Very small bacillus growing together in zoogloea. It is not motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms circular sulphur-yellow colonies. Under a low power the centre appears yellowish brown, surrounded by a clear yellow zone. The young circular as well as the older irregularly shaped colonies are coarsely granular in appearance. No liquefaction takes place.

Gelatine Tubes.—Slight granulated clouded growth on the surface and in the depth.

Remarks.—Its growth on other culture media is not recorded.

BACILLUS SUBFLAVUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Bacilli, with rounded ends, about 0·77 μ broad and from 1·5 to 3·0 μ long, consisting of several individuals hanging together. Slow motility.

Cultures.—

Gelatine Plates.—Forms small yellowish white dots in the depth. On reaching the surface the colony resembles a yellowish white shining drop, which soon becomes a flat expansion with an irregular and lobular periphery. Under a low power the surface appears lineally arranged in scales, and has a mother-of-pearl iridescence which later becomes dirty pale yellow. No liquefaction takes place.

Gelatine Tubes.—Forms a delicate greyish yellow expansion round the point of inoculation, the edge being finely serrated.

Agar-agar.—Forms a pale yellow expansion, which later becomes darker and gradually covers the whole surface. Later the colour inclines to light chrome-yellow and yellow ochre.

Potatoes.—Does not grow very abundantly, and produces a faint loamy yellow colour.

BACILLUS TREMELLOIDES

LIQUEFIES GELATINE

Authority.—Schottelius. Quoted by Tils, 'Bacteriologische Untersuchungen der Freiburger Leitungswässer,' *Zeitschrift f. Hygiene*, vol. ix., 1890, p. 315.

Where Found.—In Freiburg water, by Tils.

Microscopic Appearance.—Small bacillus about 0·75 to 1 μ long and 0·25 μ broad, with rounded ends. It is motile.

Cultures.—

Gelatine Plates.—Forms smooth-rimmed yellow dot colonies in the depth, whilst on the surface it forms small yellow projections, which later extend on all sides. Under a low power the rim is smooth, but irregularly lobular. The colony consists of small isolated heaps of bacilli, which are gathered together in aggregates.

Gelatine Tubes.—Along the needle's path in the depth innumerable small isolated colonies of a yellow or yellowish brown colour are gathered together. On the surface a growth appears resembling a surface plate-colony, and after from eight to ten days the colony gradually sinks and the gelatine becomes thick and fluid.

Agar-agar.—Forms a dry granular growth along the needle's path on the surface. After several days it becomes surrounded with a slimy yellow and shining periphery.

Potatoes.—Produces a coarsely granular, crumbly yellow protuberance. After fourteen days the growth has sunk somewhat, and is surrounded with a slimy periphery.

Remarks.—It is aërobic.

BACILLUS FLAVESCENS

Authority.—Pohl, 'Ueber Kultur und Eigenschaften einiger Sumpfwasser-bacillen und über die Andwendung alkalischer Nährgelatine,' *Centralblatt f. Bakteriologie*, vol. xi., 1892, p. 144.

Where Found.—In marsh-water, and isolated by means of ammonia gelatine. (See p. 67.)

Microscopic Appearance.—Bacillus 2·1 to 2·2 μ long and 0·8 μ broad. Slightly motile.

Cultures.—

Gelatine Plates.—Forms yellow granular pin-head colonies. It grows very slowly, the first colony appearing only after four days.

Gelatine Tubes.—Expands over the whole surface of the gelatine, and grows in the depth also.

Agar-agar.—Grows on the surface, following the course of the needle, and gives rise to isolated small yellow centres; later it spreads very slowly over the surface of the agar.

Potatoes.—It grows more rapidly on this medium, a visible growth appearing already after forty-eight hours, whilst in four days the surface is covered with a slimy yellow expansion.

BACILLUS AUREUS

Authority.—Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water, by Adametz. Eisenberg states that it is found on the skin of eczema patients. It is also probable that this organism is identical with the *B. aureus* obtained from air and described by Percy and G. C. Frankland, 'Studies on some new Micro-organisms obtained from Air,' *Phil. Trans. Roy. Soc.*, 1887, p. 272.

Microscopic Appearance.—Straight or only slightly bent fine bacilli, 1·5 to 4 μ long and 0·5 μ broad. The majority lie parallel to one another in groups; many occur in pairs or in long threads. Possesses only slight motility. No spore formation observed.

Cultures.—

Gelatine Plates.—The colonies develop slowly and are of various shapes, and even after eight days the larger number only resemble small white dots, which, however, become later of a bright yellow, and finally chrome yellow, colour. Under a low power they appear of various dimensions, sometimes circular and sometimes elongated. They are sharply defined and of a characteristic gold colour. No liquefaction of the gelatine takes place.

Gelatine Tubes.—Grows slowly on the surface, forming an irregular and raised dark chrome yellow expansion. Hardly any growth in the depth.

Potatoes.—Produces an irregular dark chrome yellow expansion; in older cultures it becomes red brown in colour.

BACILLUS FLUORESCENS AUREUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Short bacillus about 0·74 μ broad, and about twice as long, with rounded ends; occurs in pairs, rarely in greater numbers; the individuals lie together in larger and smaller groups. It is very motile, and has cilia attached. No spore formation observed.

Cultures.—

Gelatine Plates—In the depth, small yellowish white dots; on the surface, yellowish grey, moist and shining expansions. Under a low power the depth colonies are sharply defined, pale yellow, granular and smooth-rimmed discs; the surface colonies are irregular in contour, denser in the centre and delicately streaked.

Gelatine Tubes.—Forms a thin yellowish expansion, which later becomes thicker, and reaches after from eight to ten days the edge of the glass. It only grows very scantily in the depth, and assumes a somewhat brownish appearance. When streaked on sloped gelatine it produces a thickish ochre yellow expansion, beneath which the gelatine becomes strongly brown in colour, and becomes fluorescent. No liquefaction takes place.

Agar-agar.—Produces an abundant ochre or gold-yellow extensive expansion, whilst the agar becomes darker in colour, but does not fluoresce.

Potatoes.—Similar growth as on agar-agar, but less plentiful.

BACILLUS AURANTIACUS

Authority.—Percy and G. C. Frankland, *Zeitschrift für Hygiene*, vol. vi. p. 390.

Where Found.—In the deep well-water obtained from the chalk by the Kent Company.

Microscopic Appearance.—Short fat bacillus of very variable dimensions. It grows in pairs and also forms long threads. The short bacilli are nearly 1·7 μ long, and nearly half as wide as long. No spores were observed. The individual bacilli are motile.

Cultures.—

Gelatine Plates.—Produces bright orange pin-heads. Under the microscope the depth colonies are seen to be smooth-rimmed. No liquefaction of the gelatine takes place, and its growth is slow.

Gelatine Tubes.—A shining orange-coloured expansion forms on the surface, whilst hardly any growth is visible in the depth.

Agar-agar.—Forms a bright orange expansion, which does not extend much beyond the point of inoculation.

Broth.—The liquid remains clear, whilst a slightly orange-coloured deposit is produced. A thin pellicle forms on the surface, which exhibits here and there bright spots of orange colour.

Potatoes.—Produces a thick and magnificent brilliant red orange pigment, which is, however, restricted to the point of inoculation.

Remarks.—Reduces the nitrate to nitrite only very slightly when introduced into the nitrate solution. (See p. 27.)

BACILLUS AQUATILIS (Percy Frankland)

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, *Zeitschrift für Hygiene*, vol. vi. p 381.

Where Found.—Found by the above in water obtained from deep wells sunk into the chalk by the Kent Company. The *Bacillus aquatilis a* found in the Dorpat water by Tataroff, and described by him in *Die Dorpater Wasserbacterien* (Dorpat, 1891), is doubtless identical with the above.

Microscopic Appearance.—Very similar in appearance to *B. arborescens*, forming also long wavy threads, sometimes as long as 17 μ and more. No spores observed. Vibratory movement only.

Cultures.—

Gelatine Plates.—In the depth the colonies at first appear smooth-rimmed, but the contour gradually becomes more and more irregular. On reaching the surface slow liquefaction of the gelatine commences, and convoluted bands of threads extend from the centre to the periphery.

Gelatine Tubes.—Grows extremely slowly; forms a slightly yellow expansion on the surface, but hardly any growth appears in the depth. Later slight liquefaction takes place.

Agar-agar.—Produces a small shining yellow growth.

Broth.—Renders it turbid, and produces a whitish deposit. No pellicle is formed.

Potatoes.—Hardly any growth at all.

Remarks.—When introduced into the nitrate solution (see p. 27) it grows abundantly, but fails to convert the nitrate to nitrite.

BACILLUS AQUATILIS

Authority.—Lustig, *Diagnostik der Bakterien des Wassers*, 1893, p. 67.

Where Found.—Found by Lustig and Carle somewhat frequently in various waters—river, stream, spring, and stagnant water—obtained from the Aosta valley.

Microscopic Appearance.—Short, straight bacillus, three times as long as broad, with rounded ends. Occurs singly, and occasionally several bacilli hang together forming filaments. Exhibits lively pendulum-like movements. No spore formation was observed. Is coloured with the ordinary stains, but will not stain by Gram's method.

Cultures.—

Gelatine Plates.—After forty-eight hours white dots resembling mother-of-pearl appear on the surface as well as in the depth. The superficial colonies are round, and give rise to convex pin-head shaped projections. Under the microscope, with a low power, the edges look sharp and regular, and the contents granular and yellow. The colonies do not extend with age, but only become more raised. The gelatine is not liquefied.

Litmus Gelatine.—Grows similarly, but more quickly. The gelatine does not change colour even after months.

Broth.—Renders it turbid at room-temperature.

Agar-agar.—Produces a white, moist expansion, but only grows at the temperature of a room.

Potatoes.—Grey white expansion, having a smeared appearance, with irregular contour; after six days the growth becomes of a coffee-yellow colour.

Remarks.—It grows rapidly even in the absence of air, but will not de velop at a higher temperature than from 23° to 25° C. It grows luxuriantly in ammonia solutions without oxidation. It reduces nitrates to nitrites.

BACILLUS PHOSPHORESCENS GELIDUS

Authority.—Förster, 'Ueber einige Eigenschaften leuchtender Bakterien,' *Centralblatt f. Bakteriologie*, vol. ii., 1887, p. 337.

Where Found.—On phosphorescent sea-fish.

Microscopic Appearance.—In very young cultures the bacilli are small, about three times as long as broad, with slightly rounded ends. In cultures more than twenty-four hours old the rods are for the most part thickened and become egg-shaped. (Involution forms.)

Cultures.—It grows abundantly on a culture material containing 2 to 7 per cent. of common salt. A favourable medium is fish broth made with sea-water or containing from 3 to 4 per cent. of common salt. If more than 7 per cent. of salt is added the development ceases, and with 7 per cent. is already retarded.

Gelatine Plates.—At ordinary temperatures forms small round dots in from one to two days. Under a low power they are almost circular, grey white in colour, with a faint green opalescence; later they become granular, light yellow, and their periphery slightly irregular.

Gelatine Tubes.—Very slight growth in the depth; forms a white expansion on the surface. No liquefaction takes place.

Agar-agar.—Similar to gelatine-tube cultures.

Potatoes.—Forms a broad white expansion.

Remarks.—Cultivations of this bacillus give out a magnificent phosphorescence in the presence of air and when seen in the dark. This phenomenon takes place at 0° to 20° C., but ceases at 32° C. A temperature of from 35° to 37° C. destroys the bacillus, but, on the other hand, it can grow at 0° C. without much retardation.

BACILLUS PHOSPHORESCENS INDICUS

LIQUEFIES GELATINE

Authority.—Fischer, 'Bakteriologische Untersuchungen auf einer Reise nach Westindien,' *Zeitschrift f. Hygiene*, vol. ii., 1887, p. 54. See also Katz, 'Zur Kenntniss der Leuchtbakterien,' *Centralblatt f. Bakteriologie*, vol. ix., 1891, p. 158.

Where Found.—In sea-water.

Microscopic Appearance.—Small thick bacillus about two to three times as long as broad, with rounded ends. Gives rise to threads more or less bent. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—After thirty-six hours small round whitish grey dots are visible. The surface colonies give rise to liquid depressions. Under a low power the smallest centres are circular, smooth-rimmed, and of a pale bluish or sea-green colour, often exhibiting a rose-coloured hue. When liquefaction has proceeded still farther the colonies assume a brownish or dirty yellow colour. The periphery becomes wavy, and the same rosy blush is sometimes visible.

Gelatine Tubes.—Forms in about four days a depression mostly filled with air at the point of inoculation; a faint grey white, thread-shaped turbidity extends all along the needle's path in the depth. In from eight to ten days the depression has increased in size, so that a pea can easily lie in it, and the bottom and sides are covered with a dirty yellow substance. The turbidity and liquefaction below the depression have only increased very slightly. Later on a dirty yellow pellicle forms on the surface of the liquid gelatine.

Agar-agar.—Forms a grey white expansion which exhibits phosphorescence in the dark.

Potatoes.—At from 15° to 20° C. it forms a broad thin white expansion.

Blood Serum.—Grows luxuriantly, forming a grey white expansion with a lobular periphery.

Remarks.—Grows best at from 20° to 30° C.; it will not grow below 10° C., and is destroyed at 55° C. It will not phosphoresce below 0° C., and exhibits this property best when kept between 25° and 30° C. Beyerinck (see p. 453) recommends a culture material consisting of fish broth made with sea-water to which 1 per cent. of glycerine, ¼ per cent. of asparagin, and 8 per cent. of gelatine are added.

BACILLUS PHOSPHORESCENS INDIGENUS

LIQUEFIES GELATINE

Authority.—Fischer, 'Ueber einen neuen lichtentwickelnden Bacillus,' *Centralblatt für Bakteriologie*, vol. ii., 1888, p. 105. See also Katz, 'Zur Kenntniss der Leuchtbakterien,' *Centralblatt f. Bakteriologie*, vol. ix., 1891, p. 159.

Where Found.—In sea-water in the harbour of Kiel; also obtained from the bodies of green herrings. As many as from four to twenty individuals were found in 1 c.c. of the harbour water.

Microscopic Appearance.—Short fat bacillus, resembling the *B. phos. indicus* (p. 451), but somewhat shorter. Their length varied between 1·3 and 2·1 μ, and their breadth between 0·4 μ and 0·7 μ. Occurs mostly in twos; forms also threads. It is very motile.

Cultures.—Grows best on herring gelatine (*i.e.*, green herrings substituted for beef and gelatine). If a small spot of a cooked green herring is inoculated with the bacillus the whole fish becomes covered in a few days with a greyish white slimy material, which exhibits phosphorescence in the dark. By adding common salt to ordinary culture gelatine the growth of the bacillus is not only stimulated, but it becomes more phosphorescent.

Gelatine Plates.—A depression is visible after a few days in the vicinity of the bacillus, and in about seven days a thin dirty yellow disc is seen lying at the bottom of it. Under a low power the smaller colonies are circular and smooth-rimmed, of a pale sea-green colour. Older colonies have a dirty greyish yellow colour, and a pinkish tinge is usually visible near the edge of the colony. The liquefaction of the gelatine takes place more slowly than in the case of *B. phos. indicus*.

Gelatine Tubes.—In about a week a narrow funnel-shaped liquid depression is visible about 2 mm. wide, and filled with air to about a depth of 1 cm., below which are flocculent and crumbly growths surrounded by a very little liquid. The depression becomes later deeper, but not much wider, and the liquid gelatine in old cultures has almost entirely disappeared.

Potatoes and Blood Serum.—No growth.

Remarks.—The brilliancy of the phosphorescence is not so great as in the case of the *B. phos. indicus*. If some of the culture material be added to sea-water, the latter phosphoresces in the same manner as ordinary phosphorescent sea-water. It is not pathogenic.

BACILLUS ARGENTEO-PHOSPHORESCENS LIQUEFACIENS

LIQUEFIES GELATINE

Authority.—Katz, 'Zur Kenntniss der Leuchtbakterien,' *Centralblatt f. Bakteriologie*, vol. ix., 1891, p. 158.

Where Found.—In sea-water, a short distance from Sydney. Probably identical with the *Photobacterium luminosum* of Beyerinck (No. 1, *a*, *Arch. Neerland.*, vol. xxiii., 1889, p. 403). See also a careful study of phosphorescent bacteria in general, communicated by Beyerinck to the Dutch Academy of Sciences, *Transactions*, Amsterdam, 1890.

Microscopic Appearance.—Straight or slightly bent bacilli, with rounded ends, about 2·5 μ long, and about one third as wide. Stains well with Loeffler's methylene blue. Very motile in drop cultures, where it forms masses of longer and shorter threads, bent and interwoven. No spore formation observed.

Cultures.—

Gelatine Plates.—At from 17° to 20° C. small hyaline coloured discs appear in twenty-four hours on the surface, which under a low power are very finely granular and light brown in colour, with irregular and lobular periphery. The depth colonies are, under a low power, straw-yellow in colour, and their surface pitted, resembling a mulberry in appearance, the contour being wavy. In two days hollow liquid depressions enclose each colony, which sinks to the bottom. Later on a yellowish white mass, surrounded by a grey turbid band, is the appearance of the liquid colonies, whilst under a low power a zone extends from this band, and numerous minute radial hair-like threads ramify into the still solid gelatine. After being cultivated through forty-one generations the liquefaction was much retarded, the liquid depressions not appearing until about the eighth day, the colonies meanwhile forming considerably large circular and thin expansions.

Gelatine Tubes.—On surface growth resembles the appearance of typical plate-cultures of the bacillus. The liquefaction proceeds rapidly, and a yellowish stringy deposit forms at the bottom of the tube. A pellicle forms on the surface, and the liquid, although at first turbid, becomes clear. No thread-like extensions are visible at the edge of the liquid depression or in the needle's path in the depth.

Agar-agar.—Forms a whitish grey viscid expansion, which, beyond its phosphorescent properties, is not characteristic.

Broth.—Renders the liquid turbid, and produces a deposit and a pellicle on the surface. Later the liquid becomes clear.

Remarks.—It is facultatively anaërobic. Grows best at 25° C.; an exposure to 32° to 34° C. for two and a half days destroys it, whilst at 13° to 15° C. its development is retarded. Sterilised distilled water kills the bacilli in a short time (fourteen hours at 17° to 20° C.). Its phosphorescence is easily lost in cultures, but may be restored by being cultivated on cooked 'garfish.'

BACILLUS GRANULOSUS

LIQUEFIES GELATINE

Authority.—Russell, "Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift für Hygiene*, vol. xi., 1891, p. 194.

Where Found.—Obtained very frequently in sea mud near the coast, and also at a depth of 1,100 metres, where it was more abundant than any other form.

Microscopic Appearance.—In suspended gelatine drop-cultures this bacillus forms long, slender threads, the individuals composing which are rather large bacilli, with thick membrane and finely granular protoplasmic contents. In older cultures the threads break up into short irregular pieces, and the isolated bacilli are shorter, plump, and coarsely granular. It forms spores enclosed in short fat cells, which are often much broader than long. In broth cultures at 37° C. a slow swinging movement is apparent, but usually it is not motile. It is coloured by Gram's method, and will stain easily with fuchsin, Ziehl's solution, whilst with Kühne's carbolic methylene blue (see p. 46) the granular particles are more strongly coloured than the other portions of the cell.

Cultures.—

Gelatine Plates.—The surface colonies are usually thin, almost transparent, and spread out like a leaf. Under a low power the edge is smooth and irregular; concentric lines are visible, due to the parallel arrangement of the filaments; the surface is furrowed, which produces irregular light or dark lines recalling the veins in a leaf. Liquefaction soon commences, and the periphery becomes irregular and prolongations from the central mass of the colony penetrate into the surrounding gelatine. In the depth the colonies are small, round, shining and opaque. The contents of the colonies are stringy, and often a whole surface colony comes away on the point of the needle.

Gelatine Tubes.—Forms a flat liquid depression, at the bottom of which the growth collects. Later the lower layers of gelatine also become fluid.

Agar-agar.—Forms after two or three days, or at 37° C. after twenty-four hours, a sparse thin growth consisting of yellowish or whitish spots. Near the condensed water, where the agar is moister, it grows more abundantly, producing an intensely white expansion.

Potatoes.—Forms a thick shining white and stringy patch, which later becomes dull and wax-like, and becomes gradually more and more brown.

Broth.—Renders it turbid, and produces a considerable deposit.

Remarks.—Although aërobic, it will grow anaërobically, but no considerable growth takes place.

BACILLUS LITORALIS

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift für Hygiene*, vol. xi., 1891, p. 199.

Where Found.—In sea mud, but only in the vicinity of the coast.

Microscopic Appearance.—A short bacillus two to four times as long as broad, with very irregular movements. Stains readily with Loeffler's blue, but is not coloured by Gram's method. No spore formation observed.

Cultures.—

Gelatine Plates.—The depth colonies appear in three days as small brown smooth-rimmed dots. The surface colonies are at first slightly opalescent, but become shining and more extended. Under a low power they are smooth-rimmed, with fine granular contents. In from five to eight days slow liquefaction commences, and proceeds so slowly that the gelatine often evaporates, giving the appearance of the colony having eaten its way into the gelatine.

Gelatine Tubes.—After forty-eight hours an undefined line is visible all along the needle's path in the depth; in three or four days a thin, whitish irregular expansion forms at the point of inoculation on the surface, and the gelatine begins to liquefy. The evaporation of the gelatine is more rapid than its liquefaction, and a hollow funnel is formed, the bottom and sides of which are covered by thin growths of the bacillus. In the depth the growth is often reddish brown in colour, and the gelatine becomes somewhat brown. This pigment is apparently only produced in the absence of air.

Agar-agar.—Forms a thin, narrow, moist greyish white expansion.

Potatoes.—No growth.

Broth.—Renders the liquid turbid, producing a fine deposit, but no pellicle.

Remarks.—Grows also occasionally in the absence of air, but not with any certainty. In anaërobic plates colonies sometimes made their appearance, and were surrounded with a brown circle. On further cultivating these colonies they sometimes grew and sometimes refused to grow in the absence of air.

BACILLUS LIMOSUS

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift für Hygiene*, vol. xi., 1891, p. 196.

Where Found.—In sea mud near the coast, and also at a depth of 1,100 metres.

Microscopic Appearance.—A long bacillus with rounded ends, 1·25 μ broad and 3 or 4 μ long. Usually two or three individuals are joined together and exhibit a slow, quiet, oscillatory movement (gelatine cultures), but when examined from potato cultures isolated cells, shorter and fatter than those from gelatine, are found which exhibit no movement. Forms bright shining spores at one end of the rod.

Cultures.—

Gelatine Plates.—Forms in twenty-four to thirty hours almost transparent centres surrounded by a slight depression. Under a low power numerous long slender threads extend from the periphery into the adjacent gelatine; later the centre of the colony spreads out and overwhelms the filaments, and the whole colony becomes circular and opaque, with a thick border of tiny thorn-like projections. In older colonies a thin pellicle forms on the surface and flocculent particles float below, whilst the edge retains its thorny appearance.

Gelatine Tubes. If sea-water is used in the preparation of the gelatine the growth is more rapid, a funnel-shaped liquid depression forming in twenty-four hours. In seventy hours the whole contents of the tube are fluid, and in the lower portions light flocculent particles collect, whilst a thin and easily torn pellicle forms on the surface. In ordinary gelatine it grows in the same manner, only more slowly.

Agar-agar.—Grows abundantly, forming a moist, white and shining expansion.

Potatoes.—Grows well, producing a thin, clouded greyish white expansion, which spreads for some distance over the surface.

Broth.—Renders it very turbid, producing a considerable deposit and forming a thick tough pellicle on the surface.

BACILLUS HALOPHILUS

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift für Hygiene*, vol. xi., 1891, p. 200.

Where Found.—Occasionally in sea-water and sea-mud.

Microscopic Appearance.—Very variable in appearance. In recent sea-water gelatine cultures it is a small bacillus 0·7 μ broad and from 1·5 to 3·5 μ long, occurring frequently in pairs, and endowed with great motility. Later yeast-shaped and also extended forms appear, the abnormal forms becoming more numerous with the age of the culture and more pronounced in ordinary than in sea-water gelatine. No spore formation was observed. It is with difficulty stained with aniline colours; it will not stain with Loeffler's solution or by Gram's method. It only stains very unevenly with both Ziehl's and Kühne's (see p. 46) solutions.

Cultures.—

Sea-water Gelatine Plates.—Circular, greyish white, semi-transparent colonies appear slowly in the gelatine. Slow liquefaction takes place, but the liquid is rapidly evaporated and a sharply defined deep depression is left in the gelatine. Under a low power each colony appears as a white halo, but later this disappears and the liquid gelatine spreads over the surface.

Sea-water Gelatine Tubes.—It grows with great difficulty in artificial media, and then only in sea-water with ordinary gelatine. Numerous other media were tried, but without any result. In from twenty-four to thirty-six hours irregularly shaped, isolated, cloudy dots appear along the needle's path in the depth, which soon coalesce and liquefy the gelatine, producing gas sometimes in such quantities that the liquid gelatine is forced up in a frothy mass over the still solid gelatine. Later the liquid usually becomes clear, and a fine deposit collects at the bottom.

Remarks.—In cultures it produces a strong alkaline reaction. Another bacillus obtained from sea-water by Russell is described on p. 468; a spirillar form on p. 407; and a cladothrix on p. 517.

BACILLUS ZOPFII

LIQUEFIES GELATINE

Authority.—Kurth, *Botanische Zeitung*, 1883. Flügge, *Die Mikroorganismen*, 1886, p. 326.

Where Found.—In the intestine of fowls. Found by Macé (*loc. cit.*) in water and soil.

Microscopic Appearance.—Bacillus from $\frac{3}{4}$ to 1 μ broad and 2 to 5 μ long. It forms long threads in liquid culture media, but in gelatine it gives rise to threads with spiral windings and every kind of snake-like contortions. It is very motile. Forms spores.

Cultures.—

Gelatine Plates.—After forty-eight hours numerous white dots are visible, from the centre of which radiate a number of fine threads, resembling the growth of a mould. Scattered about in this network numerous small white spots are visible, which under a low power are seen to be circular brownish yellow zoogloea groups, some of which are provided with isolated knotty extensions. The centre itself consists of broad interwoven bands of parallel threads, which are sometimes straight as well as plaited. (Schedtler.)

Gelatine Tubes.—According to Roux (*Précis d'Analyse microbiologique des Eaux*, p. 334), liquefaction takes place after several weeks. A thick whitish yellow growth develops all along the needle's path in the depth, from which white radial lines extend and cross one another.

Blood Serum.—No growth.

Remarks.—It grows best at 20° C. At from 30° to 37° C. the movements of the bacilli cease, at from 37° to 40° C. involution forms appear, and after being kept for some time at this temperature the bacilli are destroyed.

BACTERIUM HYDROSULFUREUM PONTICUM

Authority.—Zelinsky, 'On Hydrosulphuric Fermentation in the Black Sea and the Limans of Odessa,' *Proceedings of the Russian Physical and Chemical Society*, vol. xxv., fasc. 5, 1893.

Where Found.—In ooze of Black Sea, from depths of 1207, 870, 389, 40 and 16 fathoms, obtained during the 'Zaporozhets' expedition, 1891.

Microscopic Appearance.—Motile elongated rod.

Cultures.—

Agar-agar.—Produces a dark coffee-coloured pigment, which blackens in contact with air and gives rise to sulphuretted hydrogen.

Special Solution.—Produces a considerable amount of sulphuretted hydrogen when cultivated for three days in the following solution: 1 per. cent. solution of ammonium tartrate, 1 to 2 per cent. solution of grape-sugar, $\frac{1}{2}$ to $\frac{1}{3}$ per cent. of hyposulphite of soda, 0·1 per cent. of potassium phosphate, and traces of calcium chloride. Ammonium tartrate may be replaced by ammonium succinate. Besides sulphuretted hydrogen, traces of ammonia and possibly amines are noticed in the cultures.

Remarks.—It cannot grow in the presence of acids; the medium, if neutral, is rendered alkaline. It grows in albuminous media, but does not require considerable amounts of albuminous matters for its growth, and can live without them under the conditions existing in the Black Sea (nearly a constant temperature of 9° C. below 100 fathoms). It lives upon the cellulose of vegetable *débris*, and abstracts the oxygen of the salts of sulphur. It will also grow in media containing no sulphur of organic origin, but sulphates and sulphites. As regards the production of sulphuretted hydrogen and of ammoniacal compounds, it resembles the 'liman microbe' (*Vibrio hydrosulfureus* of Brusilowsky), which produces sulphuretted hydrogen in even larger quantities. It will grow under both aërobic and anaërobic conditions. In all samples of this ooze cultivated in anaërobic conditions in an atmosphere of nitrogen numerous micro-organisms were found which liberated sulphuretted hydrogen, but the one here described was the most characteristic. (Prince Kropotkin kindly supplied us with the above translation from the original memoir, which appeared in Russian.)

BACTERIUM SULFUREUM

LIQUEFIES GELATINE

Authority.—Holschewnikoff, *Fortschritte der Medicin*, vol. vii. p. 202.

Where Found.—In mud from the Wiesbaden filter-beds.

Microscopic Appearance.—Fine small bacilli, with rounded ends, about 0·5 μ broad and from 1·6 to 2·4 μ long. It moves slowly.

Cultures.—

Gelatine Plates.—To obtain satisfactory cultures it is best to cover the plate with a film of sterilised oil. After forty-eight hours small, sharply circumscribed, dot-shaped colonies are visible, which on reaching the surface produce funnel-shaped slowly liquefying centres. The liquefaction proceeds so slowly that the gelatine becomes evaporated, and finally small sharply defined funnel-shaped depressions filled with air are formed.

Gelatine Tubes.—Produces small colonies in the depth, and whilst a funnel-shaped depression commences from the surface downwards, slow liquefaction takes place. In the presence of air the colour of the growth is white, but in the absence of air no liquefaction follows, and a reddish brown or red colour prevails.

Agar-agar.—At 37° C. grows fairly rapidly in the form of a slimy grey mass, in the interior of which the colour is pinkish or reddish brown.

Potatoes.—In the presence of air no growth appears; in the absence of air it forms a reddish brown expansion.

Milk.—After ten days, without any coagulation taking place, the casein is dissolved.

Remarks.—Produces sulphuretted hydrogen, according to the nature of the culture material and atmospheric conditions. It produces in sterilised urine in the absence of air large quantities of sulphuretted hydrogen.

BACILLUS RADIATUS AQUATILIS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Bacilli varying in length, about 0·65 μ broad; the shortest rods are about 1·0 μ and the longest 6·5 μ long. Only the shorter rods exhibit slight motility.

Cultures.—

Gelatine Plates.—After two days in the depth whitish blue centres are visible with irregular contour, and often having a white spot in the centre. Under a low power numerous root-like ramifications are visible, causing the colony to resemble a mould. In three days the colonies are nearly circular, and under a low power resemble small woolly balls, out of which threads extend. Finally the gelatine is broken at one point, and one or more small drops of liquid resembling water come to the surface. In four days a liquefied cup-shaped depression appears, in the midst of which a circular mass of bacterial growth, whitish yellow or cream-coloured, is visible; round this is seen a ring composed of the same material, only usually deeper in colour, and delicate rays pass from it to the edge of the depression.

Gelatine Tubes.—Forms on the surface a circular and thin expansion which often exhibits radial foldings, whilst in the depth a funnel-shaped growth is visible, and on the third day liquefaction commences. A yellowish deposit collects at the bottom, and in the turbid liquid flocculent particles are visible.

Agar-agar.—Grows slowly at first, forming a smooth, shining expansion, brownish yellow in transmitted light, and pale greenish blue in reflected light.

Potatoes.—Forms a yellow ochre, sometimes reddish brown, expansion.

BACILLUS PLICATUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply. A *Bacillus plicatus* obtained from air has been previously described by Percy and G. C. Frankland ('On some new Micro-organisms obtained from Air,' *Phil. Trans.*, vol. clxxviii., 1887, p. 273), but the organism obtained from water does not resemble it.

Microscopic Appearance.—Small thin bacillus, about 0·45 μ broad and 0·48 μ long, with rounded ends. It occurs mostly in pairs, but also in fours, and hanging together in larger numbers. It is not motile.

Cultures.—

Gelatine Plates.—In the depth the colonies are yellowish white and somewhat irregular and elongated in shape. Under a very low power distinct lobulations are visible, which increase when the colony reaches the surface, recalling a yellowish white mulberry in appearance. They are seen, when rather more magnified, to be greyish yellow, irregular in contour, and covered on the surface with innumerable small protuberances.

Gelatine Tubes.—Forms an irregular yellowish white expansion, which becomes drawn together and wrinkled. After eight to fourteen days it sinks, and the gelatine becomes gradually liquefied. In the depth an abundant growth is visible, consisting of strings of yellow-white granular heaps.

Potatoes.—Forms a thin expansion, which soon becomes dried and assumes a greyish yellow and brittle appearance.

BACILLUS NUBILUS

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, 'Ueber einige typische Mikroorganismen im Wasser u. im Boden,' *Zeitschrift f. Hygiene*, vol. vi., 1889, p. 386.

Where Found.—In filtered river Thames water. Found also by Tils (*loc. cit.*) in Freiburg water, also probably by Tataroff (*loc. cit.*) in Dorpat water. This author considers the *Bacillus nubilus* to be identical with the *Bacillus gracilis* subsequently described by Zimmermann (*loc. cit.*).

Microscopic Appearance.—Slender bacillus, about 3 μ long and 0·3 μ broad. Forms long wavy threads in broth cultures. The isolated bacilli exhibit violent rotatory movements, but the threads are quite stationary. No spores observed.

Cultures.—

Gelatine Plates.—Forms cloudy undefined patches, which under the microscope are seen to consist of a thick and tangled mass of bacillar threads. Rapid liquefaction of the gelatine takes place.

Gelatine Tubes.—The surface is liquefied, but all along the path of the needle a series of horizontal circular plates arise, having a delicate cloud-like appearance. Later the whole contents of the tube become fluid.

Agar-agar.—Produces a thin opalescent blue violet expansion, the edges of which exhibit later a distinct violet fluorescence.

Potatoes.—Forms a delicate and slightly yellow growth, which is barely visible.

Broth.—Renders it turbid and produces a dirty white deposit, whilst the surface becomes covered with a thin pellicle.

Remarks.—Reduces a very small proportion of the nitrate to nitrite. (See p. 27.)

BACILLUS LIODERMOS (Flügge)

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 323.

Where Found.—Found in water by Adametz (*loc. cit.*). A *Bacillus liodermos* (*Gummibacillus*) has been isolated by Loeffler from milk (*Berliner klin. Wochenschrift*, 1887, p. 630), but it is uncertain whether it is the same as Flügge's.

Microscopic Appearance.—Small, short bacillus with rounded ends. It is very motile.

Cultures.—

Gelatine Plates.—Forms irregularly shaped centres, which float like a small white skin on the surface of the rapidly liquefied gelatine.

Gelatine Tubes.—Rapidly liquefies the superficial layers of the gelatine, and greyish yellow flocculent particles collect, whilst lower down the needle's path exhibits a grey growth.

Potatoes.—Forms a smooth shining expansion which rapidly extends over the whole surface, giving it the appearance of having been covered with a yellowish white syrup. After some days the smooth surface becomes cloudy and slightly wrinkled, but it never produces very deep furrows like the *B. mesentericus vulgatus*.

BACILLUS LIQUEFACIENS

LIQUEFIES GELATINE

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 112. The *B. liquefaciens* described by Lustig (*Diagnostik der Bakterien des Wassers*, 1893, p. 86) resembles very closely the above.

Where Found.—In water, and occurring very frequently (Lustig).

Microscopic Appearance.—Short and rather thick bacillus with rounded ends. It is very motile.

Cultures.—

Gelatine Plates.—Forms smooth-rimmed circular colonies, with white and slimy contents. Liquefaction produces cup-shaped depressions which rapidly increase in size. After a time an odour of putrefaction is perceptible.

Gelatine Tubes.—Grows, rapidly forming a funnel-shaped depression, which towards the surface resembles an air-bubble in form. The needle's path in the depth is filled with whitish granular material.

Agar-agar.—Forms a dirty white expansion.

Potatoes.—Produces a light yellow growth.

BACILLUS LIQUIDUS

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, 'Ueber einige typische Mikroorganismen im Wasser u. im Boden,' *Zeitschrift für Hygiene*, vol. vi., 1889, p. 382.

Where Found.—Very frequently in unfiltered river Thames water.

Microscopic Appearance.—Short flat bacillus with rounded ends, occurring usually in pairs, the length of such a pair varying between 1·5 μ and 3·5 μ. The dimensions are very variable. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—It forms large cup-shaped liquefying colonies, with almost quite clear and colourless contents. Under a low power the depth colonies are smooth-rimmed and circular. When liquefaction commences the periphery becomes somewhat granular and serrated, and the colonies soon run into each other.

Gelatine Tubes.—In a few days a broad funnel-shaped depression is visible the whole length of the needle's path, containing turbid liquid and masses of flocculent material. A thin pellicle forms on the surface later, which sinks to the bottom on shaking the tube.

Agar-agar.—Grows rapidly, forming a shining smooth expansion.

Potatoes.—Produces a thick, flesh-coloured, moist expansion.

Broth.—Renders it turbid; an abundant sediment collects, and after a few days a pellicle forms on the surface.

Remarks.—Powerfully reduces the nitrates to nitrites. (See p. 27.)

BACILLUS MESENTERICUS RUBER

LIQUEFIES GELATINE

Authority.—Globig, 'Ueber einen Kartoffel-bacillus mit ungewöhnlich widerstandsfähigen Sporen,' *Zeitschrift f. Hygiene*, vol. iii. p. 322.

Where Found.—On potatoes, but also in water by various investigators.

Microscopic Appearance.—Slender bacillus with rounded ends, occurring in pairs, sometimes in fours, and so forming short threads. It is very motile. Forms lustrous egg-shaped spores. This potato bacillus is slenderer than the other potato varieties.

Cultures.—

Gelatine Plates.—After two days the depth colonies are seen, under a low power, to be circular, smooth, and yellow or brown, sometimes dark brown in colour. The surface colonies exhibit short fine threads, at first only at one spot of the periphery, but later the remainder of the periphery is composed of irregular threads, which bend and cross one another in all directions, whilst a broad light expansion consisting of delicate network, the outermost edge of which is provided with fine points, surrounds the original colony; on the fourth day liquefaction of the gelatine commences, the colony increases in size, the network disappears, and the outline of the original colony becomes indistinct, whilst light greyish brown masses are visible where the colony has expanded, which assume a sort of star-shaped arrangement. To the naked eye at this stage the colony looks bluish white, and forms liquid circular depressions. As soon as the liquefaction begins spore formation commences.

Gelatine Tubes.—Forms a flat funnel-shaped liquid depression, which soon reaches the walls of the tube and extends slowly in the depth. The fluid gelatine is slightly turbid, often contains flocculent particles, whilst a thin pellicle forms on the surface.

Potatoes.—Grows differently according to the incubating temperature. At about 15° C., on the third day, the whole surface is covered with a thin but tough and stringy, yellow, finely furrowed expansion. Spores are found on the second day. At 37° C. the surface is covered in twenty-four hours by a reddish or pink red growth, having an odour of boiled ham. In forty-eight hours the growth has spread over the sides of the potato. It grows best at 45° C., but will not develop at 50° C.

Broth.—Grows only on the surface, forming a thick tough skin, whilst beneath the liquid remains quite clear.

Remarks.—Mice and guinea-pigs were not affected by it. The spores resist three-quarters of an hour's exposure in the steam steriliser, reaching a temperature of from 109° to 113° C. They are killed by three minutes' exposure to 126° C. and two minutes to 127° C., and at 130° C. are instantly killed.

BACILLUS MESENTERICUS FUSCUS

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 321.

Where Found.—In air, in hay dust, on potato rinds, and frequently in water.

Microscopic Appearance.—Short bacillus, occurring in twos and fours. It is very motile. Forms small shining spores.

Cultures.—

Gelatine Plates.—Forms round white centres, which under a low power are brownish yellow in colour, finely granular, and smooth-rimmed. Later the rim exhibits delicate thread-like projections. The gelatine is rapidly liquefied.

Gelatine Tubes.—Forms a liquid funnel-shaped depression, whilst the needle's path in the depth is marked by a cloudy whitish appearance. In four to six days the liquefaction has extended right across the tube, and the liquid becomes filled with greyish flocculent particles.

Potatoes.—Forms a smooth yellow expansion, which rapidly becomes wrinkled, furrowed, and brown. The growth is thin and does not penetrate any distance into the substance of the potato, but it extends over the whole surface.

BACILLUS MESENTERICUS VULGATUS
('Potato Bacillus')

LIQUEFIES GELATINE

Authority.—Eisenberg (*Bakteriologische Diagnostik*, 1891, p. 117) considers the so-called 'Kartoffel bacillus' as identical with the above.

Where Found.—On potatoes, in milk, and in water, by Tils (*loc. cit.*) and other investigators.

Microscopic Appearance.—Small fat bacillus with rounded ends, in pairs, and often forming threads of four individuals. When cultivated on gelatine and agar-agar, the poles stain better than the middle of the bacillus. It is very motile. Forms large oval spores which occupy the whole interior of the bacillus.

Cultures.—

Gelatine Plates.—Forms circular yellowish colonies, which rapidly liquefy the gelatine.

Gelatine Tubes.—Liquefies the gelatine rapidly all along the needle's path in the shape of a pointed funnel; the remainder of the contents quickly become fluid, and a pellicle forms on the surface.

Agar-agar.—Produces a dirty white expansion.

Potatoes.—Grows rapidly, forming at first a moist expansion, which later becomes wrinkled and furrowed. The growth is tough and stringy.

BACILLUS INUNCTUS

LIQUEFIES GELATINE

Authority.—Pohl, 'Ueber Kultur und Eigenschaften einiger Sumpfwasser-bacillen,' *Centralblatt f. Bakteriologie*, vol. xi., 1892, p. 143.

Where Found.—In marsh-water.

Microscopic Appearance.—Bacilli about 3·5 μ long and 0·8 to 0·9 μ broad. It is motile.

Cultures.—

Gelatine Plates.—Oval or round, smooth-rimmed colonies, whitish in colour, having a shining oily appearance. The gelatine is only slightly liquefied.

Gelatine Tubes.—Forms on the surface a thick shining white expansion, and grows all along the path of the needle in the depth, radiating out at the bottom. Liquefaction only commences after several days.

Agar-agar.—Forms all along the needle's path on the surface a cloudy white growth, whilst the condensed water remains clear.

Potatoes.—Produces a slimy white expansion, which very soon covers the whole surface.

BACILLUS STOLONIFERUS

LIQUEFIES GELATINE

Authority.—Pohl, 'Kultur der Sumpfwasser-bacillen und Andwendung alkalischer Nährgelatine,' *Centralblatt f. Bakteriologie*, vol. xi., 1892, p. 142.

Where Found.—In marsh-water.

Microscopic Appearance.—Bacillus 1·2 μ long and 0·8 μ broad. Very motile.

Cultures.—

Gelatine Plates.—Circular colonies with serrated edge, lighter in colour in the centre than at the periphery.

Gelatine Tubes.—Liquefies the gelatine in the shape of a funnel. Liquefaction commences in twenty-four hours.

Agar-agar.—Forms a thick white growth along the needle streak, expanding radially at the bottom. The condensed water becomes turbid.

Potatoes.—Forms small pin-head growths which lie close together, and, starting from the point of inoculation, very soon spread over the whole surface of the potatoes.

Milk.—The milk is not coagulated, and no acid is produced.

BACILLUS INCANUS

LIQUEFIES GELATINE

Authority.—Pohl, 'Ueber Kultur und Eigenschaften einiger Sumpfwasserbacillen,' *Centralblatt f. Bakteriologie*, vol. xi., 1892, p. 192.

Where Found.—In marsh-water.

Microscopic Appearance.—Bacilli 1·7 μ long and 0·4 μ broad. Occurs usually lying parallel, two to four individual bacilli side by side. It is slightly motile.

Cultures.—

Gelatine Plates.—Forms circular granular colonies with a smooth dark rim. The gelatine is only slightly liquefied.

Gelatine Tubes.—Produces a grey white raised expansion on the surface, and grows visibly in the depth. Slight liquefaction takes place after forty-eight hours, which only progresses very slowly.

Agar-agar.—Forms grey white granular growths all along the needle's path. The condensed water remains clear.

Potatoes.—Produces an abundant and spreading grey thread-like growth.

Remarks.—When cultivated on gelatine tinted blue with litmus, the colour of the latter remains unaltered, and therefore no acid is produced.

BACILLUS IRIDESCENS

LIQUEFIES GELATINE

Authority.—Tataroff, *Die Dorpater Wasserbacterien*, Dorpat, 1891, p. 57.

Where Found.—In Dorpat water.

Microscopic Appearance.—Bacillus varying in length from 3·5 to 5·2 μ; forms also long (8·7 to 10·5 μ) bent threads. Only capable of slight oscillatory movements. Forms spores.

Cultures.—

Gelatine Plates.—Forms irregular blue, greenish yellow iridescent colonies, consisting of flakes composed of numerous tiny shining plates or scales arranged like slates on a roof. After a time a yellow central disc is visible, from which liquefaction starts. Under a low power the depth colonies are granular, shining yellow and circular discs. The surface colonies exhibit at first a pale yellow shining, ill-defined disc, which later becomes broken up into isolated windings, and surrounded by a radially folded outer zone, in which isolated concentric foldings also become visible. The appearance of the colony is very characteristic, and may be compared to the convolutions of the brain; the central windings are fine and sharply defined, and shining yellow, whilst towards the periphery they are coarser, not so sharply defined, and white.

Gelatine Tubes.—Forms a thread-like growth in the depth, and on the surface a delicate shining basin, the centre of which is yellowish and depressed, whilst the outer zone is broad, irregular and iridescent. In three to four days liquefaction commences in the centre, and a slimy yellow mass collects in the depression.

Agar-agar and Glycerine-agar.—Forms a thick, shining, wavy, moist, greenish yellow, iridescent expansion, the surface of which is pitted. Later it becomes dirty yellow and opaque. It develops less well in the incubator.

Blood Serum.—Forms a yellow moist shining expansion, which rapidly forms a gutter, and the greater part of the serum is subsequently liquefied. It will not develop in the incubator.

Potatoes.—Grows slowly at first, but later forms a raised dark honey-yellow, dry, shining, rough expansion, which afterwards becomes yellowish brown and slimy. It will not develop in the incubator.

Broth.—Renders it turbid, forming a yellow deposit. In the incubator the liquid remains clear.

BACILLUS GUTTATUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.– In the Chemnitz water supply.

Microscopic Appearance.—Short bacilli about 0·93 μ broad, and from 1·0 to 1·13 μ long, at first found singly or in pairs, but later hanging together in larger numbers. Very motile. Appears to form round spores.

Cultures.—

Gelatine Plates.—In the depth small grey white dots, which on reaching the surface resemble small bluish grey drops. Under a low power the depth colonies are bluish or greyish brown in colour, smooth-rimmed, circular, and granulated. The surface colonies are also smooth-rimmed, but the brownish colour is confined to the centre, the edge being colourless. Later the contour becomes slightly irregular sometimes.

Gelatine Tubes.—Forms a bluish white shining expansion having an irregular contour. The growth is very abundant in the depth, and appears to consist of numerous round ball-shaped colonies lying close together. The gelatine is slowly (after four weeks) liquefied.

Agar-agar.—Forms a thin grey white expansion, for the most part restricted to the inoculation line.

Potatoes.—Produces a moderately abundant dull slimy yellowish green expansion.

BACILLUS GEN. NOV.

LIQUEFIES GELATINE

Authority. - Trambusti and Galeotti, ' Neuer Beitrag zum Studium der inneren Struktur der Bakterien,' *Centralblatt für Bakteriologie*, vol. xi., 1892, p. 718

Where Found.—In drinking-water.

Microscopic Appearance.—When young the bacillus is about 3 to 5 μ long, and with somewhat flattened ends; later it reaches 8 to 9 μ in length, whilst still later elliptical rings appear in the interior of the rod, which are at first attached end to end, but which later become separated and appear as if they were free within the filament; finally the filament bursts and the elliptical forms escape. These free oval forms stain more intensely at the periphery, and are about 1·5 μ long and 0·9 μ broad. From these oval forms the bacillar stage recommences. In broth cultures kept at 37° C. for three or four days, these various stages are best watched. These oval forms behave differently from spores, and are not regarded as such by the authors. An aqueous alcoholic solution of safranin was employed for staining purposes. Both the bacillar and oval forms exhibit slow rotatory movement.

Cultures.—

Gelatine Plates—At 20° C. in forty-eight hours there appear circular grey granular colonies with slightly irregular contour, and surrounded by a liquid zone.

Gelatine Tubes.—The gelatine is rapidly liquefied, so that in twenty-four hours the whole contents of the tube are fluid.

Agar-agar Plates.—At 37° in forty-eight hours slightly raised circular colonies with serrated edge are produced, from which sometimes serpentiform processes appear which become longer the farther they are from the centre.

Agar-agar Tubes.—Grows abundantly on the surface, but scantily in the form of aggregations of dot-shaped colonies along the needle's path in the depth. On sloped agar surfaces a luxuriant growth appears after twenty-four hours, grey white in colour, rugose and brittle, which completely covers the surface.

Potatoes.—Forms along the needle streak a dirty grey, dry and raised growth.

Broth.—At 37° after twenty-four hours a grey white rugose skin forms on the surface, whilst the remainder of the liquid remains quite clear, and retains its alkalinity.

Milk.—Milk is rapidly coagulated, and the liquid portion exhibits a strongly acid reaction.

BACTERIUM GRAVEOLENS

LIQUEFIES GELATINE

Authority.—Bordoni-Uffreduzzi, *Fortschritte der Medicin*, 1886, p. 157.

Where Found.—On the epidermis between the toes. Found by Tils several times in the Freiburg water supply.

Microscopic Appearance.—Small bacilli about 0·8 μ long and nearly as broad. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms irregular greyish white spots which rapidly liquefy the gelatine, giving rise to an offensive odour resembling that from feet. Later it becomes of a greenish yellow colour.

Potatoes.—Forms grey centres with a most offensive odour.

Blood Serum.—Liquefies the serum.

BACILLUS GASOFORMANS ('Gasbildender Bacillus')

LIQUEFIES GELATINE

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 107.

Where Found.—In water. Found also by Tils (*Zeitschrift für Hygiene*, vol. ix. p. 315) in Freiburg water.

Microscopic Appearance.—Small, very motile bacilli. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms rapidly cup-shaped, liquefying depressions. Under a low power the contents are seen to be greyish, and bubbles of gas are often visible.

Gelatine Tubes.—Rapidly liquefies the gelatine, producing along the needle's puncture a stocking-like depression, whilst in the still solid gelatine bubbles of gas appear the whole length of the needle's path. It will not grow at higher temperatures.

Potatoes.—Grows rapidly, producing a dark yellow, later reddish brown, slimy expansion (Tils, *loc. cit.*).

BACILLUS THALASSOPHILUS

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift für Hygiene*, vol. ix., 1891, p. 190.

Where Found.—Obtained frequently in anaërobic cultures of sea-mud.

Microscopic Appearance.—A slender bacillus of variable length, having a tendency to form filaments. It is capable of slow movements. Forms spores at the end of the rod. Will not stain with Loeffler's solution or fuchsin, but young cultures stain well with Ziehl's solution; old cultures, however, take up but little even of this stain.

Cultures.—

Sea-water Gelatine Tubes.—In from two to three days colonies in the shape of small clouded bubbles appear at the bottom of the needle's path in the depth; above these other colonies make their appearance, until finally a long, grey, semi-transparent, liquid, sack-shaped mass is formed, in the upper portions of which gas collects. The gelatine is rapidly liquefied, although much more slowly towards the surface. This liquefaction is produced by a peptonising ferment elaborated by the bacillus, but extending beyond the limits of its growth. The turbid fluid becomes subsequently clear.

Gelatine Plates.—When an ordinary gelatine plate is covered by another glass plate the colonies appear in from two to three days. Under a low power they exhibit a very thin network composed of filaments, which penetrate the gelatine, but do not give rise to any definite shape. A great deal of gas forms in the colonies, and on raising the superficial plate an intense smell of skatol is given off. The isolated colonies soon coalesce, and the whole of the gelatine becomes fluid.

Agar-agar.—Grows very slowly in the depth at a distance of 2 cm. from the surface.

BACILLUS 'AMYLOZYME'

Authority.—Perdrix, 'Sur les Fermentations produites par un Microbe anaérobie de l'Eau,' *Annales de l'Institut Pasteur*, vol. v., 1891, p. 287.

Where Found.—Very frequently in the water supply of Paris derived from the Seine and from the Vanne.

Microscopic Appearance.—Bacillus about 2 to 3 μ long and 0·5 μ broad, with rounded ends. Occurs in pairs and chains. It is motile, but the movements become slower with the increasing length of the chain. It is anaërobic, and in the presence of air its motility is diminished and even entirely checked. It forms spores, which will withstand ten minutes at 80° C.

Cultures.—

Anaërobic Gelatine Tubes.—At the end of from five to six days it forms small white centres, round each of which are seen bubbles of gas which break up the gelatine, but without causing any liquefaction.

Potatoes.—After some days whitish isolated spots are visible, which become larger and more circular, forming small nipple-shaped protuberances, in the vicinity of which the potato becomes depressed. The potato becomes partially liquefied. On opening the tubes containing the cultures there is a slight explosion, and in consequence of the diminished pressure the colonies part with the gas which they enclosed, each exhibiting tiny craters from which bubbles of gas escape.

Broth.—It grows in this medium for a short time.

Remarks.—It grows best at about 35° C.; it will produce gas at 16° to 17° C. and up to 43° C., but it will not grow at from 44° to 50° C. It ferments glucose, saccharose, lactose, starch.

BACILLUS GLAUCUS

LIQUEFIES GELATINE

Authority.—Maschek, *Die Bakterien der Nutz- und Trinkwässer*, Adametz. Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Fine slender bacillus of variable length. It is not motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Forms circular, grey colonies. Under a low power they are smooth-rimmed, and the centre is intensely grey, whilst towards the periphery they are brown and have a radial folded appearance. On the eighth day liquefaction begins, and the colony sinks.

Gelatine Tubes.—Grows quickly on the surface and in the depth, rapidly liquefying the gelatine and forming masses of grey material.

Agar-agar.—Grows quickly, producing a grey expansion.

Potatoes.—Forms a dirty white, later dark grey, expansion.

BACILLUS FULVUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Short bacilli with rounded ends, about 0·86 to 1·3 μ long and about 0·77 μ broad, consisting of one or more individuals. No spore formation observed. It is not motile.

Cultures.—

Gelatine Plates.—In the depth the colonies are irregularly rounded or egg-shaped, yellowish grey and granular. The surface colonies are of a reddish yellow colour, and after eight days have extended for a distance of 1 mm. or more; they resemble raised round drops.

Gelatine Tubes.—Produces an irregular raised circular growth resembling the colour of gamboges. The path of the needle is clearly marked and of a slightly yellow colour. Liquefaction takes place after some weeks.

Agar-agar.—Forms an abundant shining yellowish expansion.

Potatoes.—Develops slowly and produces an Indian-yellow coloured growth, which later is nearly ochre yellow.

Remarks.—It grows most rapidly at 30° C.

BACILLUS FILIFORMIS

LIQUEFIES GELATINE

Authority.—Tils, 'Bakteriologische Untersuchung der Freiburger Leitungswässer,' *Zeitschrift f. Hygiene*, vol. ix., 1890, p. 317.

Where Found.—Found in the Freiburg water supply during the autumn months. Roux (*Précis d'Analyse microbiologique des Eaux*, 1891, p. 315) says that he isolated from the river Saône at Lyons a bacillus which had a striking resemblance to Tils' form, and which he has called provisionally *Streptobacille*.

Microscopic Appearance.—Bacillus 4 μ long and about 1 μ broad; occurs usually hanging together, forming filaments consisting of as many as eight individual bacilli, the divisions being almost undistinguishable. The isolated bacilli, and those in twos or threes, have an oscillatory movement, whilst the long threads are motionless. Shining spores appear in the middle of the rod.

Cultures.—

Gelatine Plates.—The depth colonies are whitish, finely granular, with an irregular edge. The surface colonies form at the end of three days whitish expansions having a striped appearance. Under a low power the periphery is irregular and serrated, and consists of closely-packed wavy bundles of bacilli; the centre looks uneven and granular. The edge of the colony is colourless, but towards the middle it becomes yellowish. Slow liquefaction of the gelatine takes place after several days.

Gelatine Tubes.—Grows scantily in the depth, but produces a moist expansion on the surface, with a wavy serrated edge. Liquefaction commences after a few days, and thick flocculent masses sink to the bottom. Below the line of liquefaction the still solid gelatine looks very clouded.

Agar-agar.—Grows rapidly, producing a more pronounced wavy-looking growth than on gelatine.

Potatoes.—Forms a thick irregular dirty white expansion, which later becomes darker and discoloured.

BACILLUS DIFFUSUS

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, 'Ueber einige typische Mikroorganismen im Wasser und im Boden,' *Zeitschrift für Hygiene*, vol. vi., 1889, p. 396.

Where Found.—Originally in soil, found also by Tataroff (*Die Dorpater Wasserbakterien*, Dorpat, 1891, p. 58) in water.

Microscopic Appearance.—A thin slender bacillus about 1·7 μ long and 0·5 μ broad; occurs singly and frequently in pairs, and occasionally gives rise to long wavy threads. No spore formation observed. Lively oscillatory and rotatory movements.

Cultures.—

Gelatine Plates.—The surface colonies are visible as thin bluish green expansions which spread a long way over the gelatine. Under a low power the depth colonies appear almost circular, with a somewhat serrated edge and coarse granular contents. As the colony gets older the edge becomes more and more irregular. In the surface colonies the centre is less sharply defined, and becomes surrounded by a large very thin mottled expansion with a serrated and lobular periphery.

Gelatine Tubes.—The growth is almost entirely restricted to the surface, which becomes covered with a smooth, thin, shining greenish yellow expansion. The gelatine becomes very slowly liquefied and is thereby rendered turbid.

Agar-agar.—Forms a very thin smooth shining expansion, faint yellow or of a cream colour.

Potatoes.—Produces a thin faint greenish yellow smooth shining expansion.

Broth.—Renders it turbid and forms a greenish yellow deposit, whilst flocculent particles float on the surface; but no pellicle is formed.

Remarks.—Slightly reduces the nitrates to nitrites. (See p. 27.)

BACILLUS DEVORANS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In a much-used well-water.

Microscopic Appearance.—Short bacillus with rounded ends, about 0·74 μ long and 0·99 to 1·5 μ broad, occurring usually singly or in pairs, rarely in larger numbers. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—In the depth the colonies resemble small white balls; when on the surface the gelatine becomes conically depressed, and the colony appears as a round irregular white, but not homogeneous, mass at the bottom. Under a low power the latter looks granular and yellow grey in colour, whilst all round threads varying in length extend from the periphery.

Gelatine Tubes.—In the depth there is a growth visible on the next day along the path of the needle, the upper portion of which on the second to third day is filled by a more or less long bubble of air, whilst lower down white masses of material make their appearance. The gelatine becomes more and more cleft, without any trace of liquefaction, until finally the white growths accumulate on the bottom and sides of the now enlarged path of the needle. Occasionally liquefaction takes place.

Agar-agar.—Forms a thin and evenly distributed grey expansion, which in from two to three days has spread over the whole surface.

Potatoes.—No growth.

BACILLUS JANTHINUS (Zopf), (B. violaceus)

LIQUEFIES GELATINE

Authority.—Zopf, *Die Mikroorganismen*, Flügge, 1886, p 291. Described also by Plagge and Proskauer, *Zeitschrift f. Hygiene*, vol. ii., 1887, p. 463, and by Zimmermann (*loc. cit.*). The *B. violaceus* described by Macé (*Ann. d'Hyg. publ. et de Méd. lég.*, vol. xvii., 1887), the *B. violaceus* described by Percy Frankland (*Zeitschrift f. Hygiene*, vol. vi., 1889, p. 394), and the *B. violaceus laurentius* described by Jordan (State Board of Health, Massachusetts: *Purification of Sewage Water*, 1890, p. 838) deviate but slightly in the descriptions given from the above.

Where Found.—In water by various investigators, but not occurring frequently. Found also in hail by Bujwid (*Centralblatt f. Bakteriologie*, 1888, vol. iii. p 1).

Microscopic Appearance.—Longer and shorter rods about 0·65 μ broad and from 1·5 μ to 3·5 μ long. The longer rods are often slightly bent. Rotatory and vibratory movements. No spore formation observed. In the form obtained originally from Berlin and described by Percy Frankland spores were observed. Fraenkel (*Grundriss der Bakterienkunde*, 1887, p. 184) mentions the presence of spores in the *B. violaceus* obtained from the Berlin water.

Cultures.—

Gelatine Plates.– In the depth they are small white dots, but on the surface they form small bluish grey, almost circular discs, which towards the centre are slightly raised ; after five days they appear as shining drop-like, greyish yellow expansions, reaching about 2·5 mm. across. Under a low power the depth colonies are nearly circular, with a sharp contour, and yellowish brown in colour, which decreases in intensity towards the periphery. When they become older delicate concentric rings are visible in the colony. The surface colonies are pale yellow brown, which becomes so faint towards the periphery that it is with difficulty distinguished from the adjacent gelatine. The edge is very slightly lobular and serrated. (See Plate II. 2A, depth colony, magnified 100 times ; 2B, showing commencement of liquefaction, magnified 100 times ; 2C, later stage of liquefaction, magnified 100 times.) (Percy Frankland.)

Gelatine Tubes.—Forms a white expansion which gradually, from the centre outwards, becomes of a violet blue colour. After a while the growth sinks and the gelatine becomes slowly liquefied. No blue colour is visible in the depth.

Agar-agar.—Fairly abundant yellow or brownish white expansion, which after some days, or longer, becomes of a deep violet colour.

Potatoes.—Forms a violet black expansion, which gradually covers nearly the whole surface. The form described by Percy Frankland grows very badly on this medium.

BACILLUS BEROLINENSIS INDICUS ('Indigoblauer Bacillus')

Authority.—Claessen, 'Ueber einen indigoblauen Farbstoff erzeugenden Bacillus aus Wasser,' *Centralblatt f. Bakteriologie*, vol. vii., 1890, p. 13.

Where Found.—Unfiltered river Spree water.

Microscopic Appearance.—Fine slender bacillus with rounded ends, much resembling in its dimensions the typhoid bacillus. It occurs mostly singly, but also in pairs and threes, and is sometimes found, especially in fresh cultures, lying together lengthwise in packets. The bacillus is surrounded by a delicate envelope of protoplasm, which is easily seen when it is mordanted and subsequently stained. It is very motile. No spore formation observed, although bright and shining granules were noted in broth and potato cultures, but it was not possible to stain them.

Cultures.—

Gelatine Plates.—Forms pin-head colonies at first greyish white, but on the fourth day the centre becomes indigo in colour. The colourless edge of the surface colonies becomes irregular and recalls the appearance of typhoid colonies. In the depth the rim of the colony first becomes tinted, whilst the centre remains mostly greyish yellow in colour. No liquefaction takes place.

Gelatine Tubes.—At the point of inoculation after twenty-four hours a spot of deep indigo blue is seen, which increases in size, spreading gradually with irregular contour over the surface. The colour does not penetrate into the gelatine.

Agar-agar.—Produces a thick moist and shining expansion of a deep indigo blue.

Potatoes.—On *acid* potatoes it produces a deep blue expansion, on *alkaline* potatoes it gives rise to a thin moist and shining dirty green growth.

Broth.—Renders it turbid, and flocculent particles pervade the liquid. No colour is produced.

Distilled Water.—Culture material containing the bacillus when inoculated into 1 c.c. of sterilised distilled water induced in the latter a distinctly milky turbidity in twenty-four hours.

Remarks.—It is strictly aërobic. The production of pigment takes place also when the cultures are kept in the dark. It flourishes better at about 15° C. than at higher temperatures.

BACILLUS MEMBRANACEUS AMETHYSTINUS

LIQUEFIES GELATINE

Authority.—Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 421.

Where Found.—In well-water of Spalato (Jolles).

Microscopic Appearance.—Short bacilli with rounded ends, on the average from 1·0 to 1·4 μ long and 0·5 to 0·8 μ broad. They are irregularly aggregated together, and some individual bacilli stain more strongly at the ends than in the middle. They are not motile. Spore formation uncertain.

Cultures.—

Gelatine Plates.—In two to three days on a thickly sown plate small homogeneous dark violet centres make their appearance, which in the course of the next few days become surrounded by a zone of liquid gelatine. In from one to two weeks the whole of the gelatine is fluid, and the colonies which have hardly increased in size float in the clear liquid. When fewer colonies are present on the plate they give rise in three to four days to yellowish white expansions with a jagged edge, resembling the typhoid colonies; the gelatine in the vicinity of the expansion only becomes softened after from ten to fourteen days. The colony meanwhile has become deep violet in colour, and after from three to four weeks it floats as a large violet pellicle on the slightly liquid gelatine, resembling a membrane stained with gentian violet.

Gelatine Tubes.—Forms a superficial yellowish white expansion with a jagged edge, which after ten to fourteen days, or even longer, becomes violet in colour, which starts from the centre. Liquefaction only gradually commences. After about four weeks a thick dark violet pellicle covers the sunken and softened gelatine, whilst the whole contents of the tube do not become fluid until after from two to three months.

Agar-agar.—Exhibits after twenty-four hours a thick yellowish white milky expansion, which only assumes a violet colour after eight to ten days have elapsed. In three to four weeks it has grown into a very wrinkled expansion of a magnificent deep violet colour, having a metallic lustre. The agar beneath remains uncoloured, and the growth is easily removed from its surface.

Potatoes.—Grows very slowly, forming a dirty yellow to olive-green coloured expansion.

Broth.—Grows very slowly; after some weeks a violet deposit is formed, and a violet pellicle is produced on the surface of the dark brown-coloured liquid.

Remarks.—It grows only at 15° to 20° C.; no growth takes place at 37·5°.

BACILLUS CAERULEUS

LIQUEFIES GELATINE

Authority.—Smith, 'A new Chromogenic Bacillus,' *Medical News*, 1887, vol. ii., No. 27, p. 758.

Where Found.—In water from the river Schuylkill.

Microscopic Appearance.—A bacillus 2 to 2·5 μ long and ·5 μ broad; frequently forms leptothrix-like threads.

Cultures.—

Gelatine Plates.—Forms cup-like liquid depressions. No colour is visible in the depth of the gelatine, but the surface colonies exhibit a faint blue tint.

Potatoes.—Grows at the ordinary temperature, producing a beautiful dark blue growth, which later becomes an intense blue black. It only grows on the surface of the potato.

Remarks.—The pigment is contained in the cells, and is not extracted by water, alcohol, or acids. The bacillus is not pathogenic.

BACILLUS DENTRITICUS

LIQUEFIES GELATINE

Authority.—Bordoni-Uffreduzzi, *Précis d'Analyse microbiologique des Eaux*, G. Roux, 1892, p. 312. Also *Diagnostik der Bakterien des Wassers*, Lustig, 1893, p. 99.

Where Found.—In drinking water supplied to Turin.

Microscopic Appearance.—A short bacillus with rounded ends, forms zoogloea masses in young cultivations made up of eight to thirty and even more individual bacilli. It is 0·85 to 2·08 μ long and 0·50 to 0·85 μ broad. Capable of lively oscillatory movement on its own axis. No spores are produced. Stains best with aqueous alcoholic solutions of gentian and fuchsin.

Cultures.—

Gelatine Plates.—From a slightly raised central point eight or ten branches from 2 to 3 mm. broad extend, which soon divide up into other branches, and so on until they give rise to a colony which in shape resembles a tree agate. The whole is shining, moist, and of a whitish colour, but is more distinctly so in the centre and other parts of the colony where the growth is thickest. The substance of the colony is slightly viscid, which is apparent on touching it with a needle. In old cultures (forty to sixty days) the gelatine becomes softened in the middle of the colony, and gradually the whole becomes liquefied.

Gelatine Tubes.—On the surface there appears a raised circular moist white growth with a sharp contour, whilst in the depth appear numerous round whitish colonies which are confluent. After some time the gelatine becomes soft, and is finally liquefied.

Agar-agar Plates.—When preserved at 22° C. there appears a thin dirty white expansion, starting from a central point and spreading out with an irregularly serrated edge. No growth takes place at 37° C.

Agar-agar Tubes.—At 22° C. it grows much in the same way as on gelatine, but at 37° C. an abundant development takes place in the depth, whilst a fine almost invisible growth appears on the surface.

Blood Serum.—Hardly any growth at 37° C. At 22° C. it develops more abundantly in the depth than on the surface. After five to six days, however, a smooth white expansion with a delicately serrated edge forms. In old cultures the serum is liquefied.

Broth.—At 22° C. it renders it turbid, and forms a white pellicle with a rough moist surface. This becomes so firmly attached to the walls of the tube that the latter can be turned upside down without spilling the contents. At 37° C. it becomes slightly turbid and no pellicle is formed. In glycerine broth at 37° C. a pellicle is formed, but it is less tough and always sinks to the bottom of the tube.

Potatoes.—At 22° C. produces a thick white moist and shining expansion which spreads over nearly the whole surface of the potato. In old cultures the growth becomes yellow. At 37° C. the growth is more restricted.

BACILLUS CUTICULARIS

LIQUEFIES GELATINE

Authority.—Tils, 'Bacteriologische Untersuchung der Freiburger Leitungswässer,' *Zeitschrift für Hygiene*, vol. ix., 1890, p. 316.

Where Found.—In the Freiburg water supply.

Microscopic Appearance.—Slender bacillus 0·3 to 0·5 μ broad, 2 to 3 μ long. Forms threads in culture media. Very slightly motile.

Cultures.—

Gelatine Plates.—Under a low power the depth colonies appear as smooth-rimmed, irregular brownish discs. The surface colonies are at first smooth-rimmed with a lobular periphery, yellowish brown in the centre, but colourless towards the edge. The colony projects slightly above the gelatine, but after a few days it sinks and the latter is quickly liquefied, whilst the colony floats on the surface as a grey white pellicle.

Gelatine Tubes.—The gelatine is rapidly liquefied and a pellicle forms on the surface.

Potatoes.—Grows slowly, producing at first a bright yellow and slimy irregular expansion, which later becomes dark yellow.

Broth. Renders it quickly turbid in the upper parts of the tube; later the whole contents become turbid, and a pellicle forms on the surface.

BACILLUS D. (Foutin)

Authority.—Foutin, 'Bakteriologische Untersuchungen von Hagel,' *Centralblatt für Bakteriologie*, vol. vii., 1890, p. 373.

Where Found.—In hailstones.

Microscopic Appearance.—Bacillus about 1 μ broad and 5 μ up to 20 μ long; it is thinner at the poles, which are slightly rounded. Individual bacilli contain sometimes from one to four spores. The bacilli are slightly motile. They stain readily with all aniline colours.

Cultures.—

Gelatine Tubes.—Forms a nail-head growth on the surface, and has a granulated appearance in the depth resembling the erysipelas coccus. No liquefaction takes place.

Agar-agar.—Fairly abundant sharply defined growth with a mother-of-pearl iridescence.

Potatoes.—A somewhat raised yellow streak restricted to the line of inoculation, with a sharply defined edge.

Remarks.—Not pathogenic to animals.

BACILLUS C. (Foutin)

LIQUEFIES GELATINE

Authority.—Foutin, 'Bakteriologische Untersuchungen von Hagel,' *Centralblatt für Bakteriologie*, vol. vii., 1890, p. 373.

Where Found.—In hailstones.

Microscopic Appearance.—Slender bacillus about 1 to 2 μ long, resembling the *B. murisepticus*. Those obtained from potato cultures are rather thicker. It occurs singly and in chains. Forms spores.

Cultures.—

Gelatine Plates.—The colonies appear as white dots; under a low power they look bright yellow, transparent, with a finely serrated edge.

Gelatine Tubes.—Slowly liquefies the gelatine in a funnel-shaped depression; the liquid turns a brownish red colour, and a pellicle forms on the surface.

Agar-agar.—Forms a considerable pale brown and shining expansion.

Potatoes.—Grows as on agar-agar; the colour is however stronger, and later becomes dark red brown and almost black.

Remarks.—Is not pathogenic to animals.

BACILLUS CARNICOLOR ('Fleischfarbiger bacillus')

LIQUEFIES GELATINE

Authority.—Tils, 'Bakteriologische Untersuchung der Freiburger Leitungswässer,' *Zeitschrift f. Hygiene*, vol. ix., 1890, p. 316.

Where Found.—In water supplied to Freiburg during the autumn months.

Microscopic Appearance.—Slender bacillus about 2 μ long and 0·5 μ broad. Occurs usually singly. It is very motile. No spore formation observed.

Cultures.—

Gelatine Plates.—In two days round white colonies appear which form cup-shaped liquefying depressions. Under a low power the circumference is sharply defined, and around the dark centre several light and dark circlets are visible.

Gelatine Tubes.—Grows rapidly all along the needle's path, and forms a funnel-shaped liquefying depression, the lower part of which becomes filled with faint pink-coloured masses of material, after which the liquefaction ceases.

Potatoes.—Grows slowly, producing a dark flesh-coloured expansion.

BACILLUS ACIDI LACTICI

Authority.—Hueppe, *Mitth. a. d. kais. Gesundheitsamte*, vol. ii., p. 337.

Where Found.—In sour milk. Found by Adametz in water, and also by Tils (*Zeitschrift für Hygiene*, vol. ix. p. 291).

Microscopic Appearance.—Short and plump bacilli, from 1 to 1·7 μ long, 0·3 to 0·4 μ thick, half as wide as long; occurs usually in pairs, rarely in chains of four. Not motile. Produces spores at the poles. Is stained with methyl violet, methylene blue, or Bismarck brown (Hueppe).

Cultures.—

Gelatine Plates.—Small white dots to begin with; later grey white shining centres, resembling bits of porcelain, and having a slightly irregular edge. Under the microscope the surface colonies resemble small leaves spread out, the centre of which are yellowish in colour, whilst the edge is thin and finely serrated.

Gelatine Tubes.—Along the needle track in the depth are seen tiny centres, whilst later there appears on the surface a grey white shining, somewhat thick and dry expansion. Does not liquefy the gelatine.

Potatoes.—Yellow brown expansion.

Milk.—Renders it acid. Precipitation of the casein. Produces gas. No alcohol is produced.

Remarks.—It will not grow below 10° C. in milk, requires from 10° to 12° C. At 15° C. acid is produced, which ceases between 45·3° and 45·5° C. (After eight days the casein is separated out.) It will grow in the absence of oxygen. It is aërobic and facultatively anaërobic.

BACILLUS LACTIS CYANOGENUS ('Bacillus der blauen Milch')

Authority.—Hueppe, 'Untersuchungen über die Zersetzungen der Milch durch Mikroorganismen,' *Mittheilungen a. d. kaiserlichen Gesundheitsamte*, vol. ii., 1884, p. 355 Also Neelsen, 'Studien über blaue Milch,' *Cohn's Beiträge zur Biologie der Pflanzen*, vol. iii., Heft 2, 1880, p. 187.

Where Found.—In blue milk. Found frequently in Lawrence sewage by Jordan (*loc. cit.*).

Microscopic Appearance.—Bacilli about 1 to 4 μ long and 0·3 to 0·5 μ broad, with slightly blunted corners. It is very motile. Forms spores at the end of the rod.

Cultures.—

Gelatine Plates.—Forms finely granular dirty white circular colonies, with a smooth rim. After a time the gelatine in the vicinity becomes slightly darkened. No liquefaction takes place.

Gelatine Tubes.—Forms a nail-head growth, milk-white in colour; the surrounding gelatine becomes a diffused greyish blue colour, which later turns darker and almost black.

Agar-agar.—Forms a greyish expansion, the agar becoming dark brown in colour.

Potatoes.—Forms a restricted yellowish growth, whilst the whole potato assumes a diffused greyish blue tone.

Blood Serum.—Grows, but does not produce any pigment.

Remarks.—On being introduced into raw milk it produces grey blue or sky-blue spots of colour. The colour is most pronounced on the surface. Sterilised milk, when inoculated with the bacillus, does not become so intensely sky-blue in colour as raw milk; it is never coagulated, neither does it become acid; but, on the contrary, it turns gradually slightly alkaline and remains fluid. (Hueppe, *loc. cit.*, p. 358.)

BACILLUS BUTYRICUS

LIQUEFIES GELATINE

Authority.—Hueppe, 'Untersuchungen über die Zersetzungen der Milch durch Mikroorganismen,' *Mittheilungen a. d. kais. Gesundheitsamte*, vol. ii., 1884, p. 309.

Where Found.—In milk; also in water by various investigators.

Microscopic Appearance.—More or less long bacilli, sometimes bent; also forms threads. Mean dimensions, 2·1 μ long and 0·38 μ broad. It is very motile. Forms at about 30° C. shining egg-shaped spores in the middle of the rod.

Cultures.—

Gelatine Plates.—Forms in the depth small yellow aggregates, which rapidly liquefy the gelatine, the colonies running together and forming a granular brown mass. Further observation of the colonies is impossible.

Gelatine Tubes.—Rapidly liquefies the gelatine, produces a yellowish colour, and a thin, delicately folded looking pellicle, grey white in colour, forms on the surface; the liquid portion becomes cloudy and opaque.

Agar-agar.—Forms a slight and dirty yellow expansion.

Potatoes.—Produces a fawn-coloured transparent growth, which later becomes clouded, and sometimes exhibits tiny folds (Loeffler).

Remarks.—It grows best between 35° and 40° C., less well at 30° C. Produces a bitter taste in milk and its coagulation.

BACILLUS LACTIS VISCOSUS

Authority.—Adametz, *Landwirthschaftliche Jahrbücher*, 1890, p. 185.

Where Found.—In river-water near Vienna receiving the waste water from some factories. Especially in the Petersbach, where as many as from 100 to 200 per c.c. were detected. Also found in thick milk.

Microscopic Appearance.—Short rods, easily mistaken for slightly elongated cocci; surrounded by a capsule, which is particularly noticeable in milk cultures. In this medium it is nearly as broad as long, about 1·5 μ long and 1·25 μ broad, or 1·05 μ long and 0·8 μ broad. It gives rise sometimes to filaments composed of 3 to 6 individuals (milk). In old milk cultures it gives rise to involution forms resembling budding yeast-cells. It is slightly motile in young cultures.

Cultures.—

Gelatine-glycerine Plates.—Forms at the end of three or four days small dot-shaped colonies, which rapidly extend over the gelatine. They are irregular in contour, with a serrated edge and a thick whitish centre; the surface is smooth, whilst the periphery is thin and almost transparent, with a distinct opalescence. The depth colonies only grow very feebly. No liquefaction takes place.

Agar-agar.—Produces a narrow whitish growth, the edge of which is at first smooth, but becomes later finely serrated.

Milk.—Sterilised milk becomes in four to six weeks viscous like honey, so that it can be drawn out into long strings; in unsterilised milk the cream only becomes stringy, and such cream yields a white, greasy butter, which rapidly spoils, owing to the numbers of the butyric acid bacillus present. Adametz concludes that the *B. lactis viscosus* paves the way for the activity of the butyric ferment.

Remarks.—It grows rapidly at 28° C., but only moderately well at 10° to 15° C. It renders milk viscid and stringy in four weeks, due apparently to the swelling of the capsules surrounding the bacillus, and not to any fermentation process.

B. ALBUS PUTIDUS ('Fauliger weisser Bacillus')

LIQUEFIES GELATINE

Authority.—Maschek, *Die Bakterien der Nutz- und Trinkwässer*, Adametz Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Small bacillus forming threads. It is motile. No spore formation observed.

Cultures.—

Gelatine Plates.—Round white colonies, which do not extend to any distance over the surface. Under a low power they appear light brown in colour, surrounded by a bright zone, which is about 5 mm. broad at the end of five days. Liquefies the gelatine rapidly all along the path of the needle. It gives rise to an intensely putrid smell.

Agar-agar.—Produces an expansion, but with nothing characteristic.

Potatoes.—Grows rapidly, producing a slimy expansion.

'WHITE BACILLUS' (Maschek)

LIQUEFIES GELATINE

Authority.—Maschek, *Die Bakterien der Nutz- und Trinkwässer*, Adametz, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Short bacillus with rounded ends.

Cultures.—

Gelatine Plates.—Circular colonies with a white centre. Under a low power they look bright yellow. The gelatine is liquefied in three days.

Gelatine Tubes.—Forms a funnel-shaped depression already after two days, and at the end of four days the whole of the gelatine is liquid.

Agar-agar.—Grows rapidly, without any characteristic appearance.

Potatoes.—Produces a white expansion, which later (four weeks) becomes brown in colour.

BACILLUS CRASSUS AROMATICUS

LIQUEFIES GELATINE

Authority.—Tataroff, *Die Dorpater Wasserbacterien*, Dorpat, 1891.

Where Found.—In well-water in Dorpat.

Microscopic Appearance.—Fat double bacillus having a distinct compression, 3·5 to 5·0 μ long and 1·5 μ wide, with rounded ends. The poles stain more strongly than the centre. Produces also variously bent, and also long threads. Gives rise to round spores on agar-agar cultures.

Cultures.—

Gelatine Plates.—Forms cup-shaped liquefying colonies in the centre, at the bottom of which is seen a small white pellicle, somewhat resembling a rosette in appearance, surrounded by a greyish round circle with radial streaks. With a low power the depth colonies are yellow brown discs, with fine granular contents; the periphery is broken up into large rounded lappets, resembling the petals of a flower. The surface colonies are yellow brown in the centre, and are filled with granular contents; the edge is paler in colour, and is not at first easily distinguished from the adjacent gelatine; but later the periphery exhibits short and fine hairy extensions.

Gelatine Tubes.—Produces a circular white expansion, greyish in colour towards the edge, which has a radially streaked appearance. The liquefaction proceeds rapidly in a funnel-shaped depression, and a white pellicle forms on the surface which later on sinks to the bottom. A dirty white deposit is produced, and a fresh pellicle is formed on the surface.

Agar-agar and Glycerine-agar.—Forms a white shining slimy and thin expansion, and a white deposit.

Blood Serum.—Produces a narrow milk-like streak, at the bottom of which drops collect, which gradually sink down into the condensed water at the bottom of the tube; but they remain isolated, and do not combine either with the water or each other.

Broth.—Renders it turbid, with a dirty white deposit.

Potatoes.—A light brown smooth and shining growth soon covers the whole surface.

Remarks.—The above name is given to this organism on account of the fatness of the bacilli and the fruit-like odour given off by the gelatine-plate cultures.

BACILLUS AQUATILIS GRAVEOLENS

LIQUEFIES GELATINE

Authority.—Tataroff, *Die Dorpater Wasserbacterien*, Dorpat, 1891, p. 48.

Where Found.—In Dorpat water. It has many points of resemblance with the *B. graveolens* isolated from between the teeth, and described by Bordoni-Uffreduzzi. (Eisenberg, *Bakteriologische Diagnostik*, 1891, p. 108.)

Microscopic Appearance.— Slender thin bacillus about 1·3 μ long. On potatoes short bent threads are visible. Slightly motile.

Cultures.—

Gelatine Plates.— Circular colonies having a yellowish centre and grey periphery. Under a low power the surface colonies are pale yellow and mulberry-shaped; later, when liquefaction commences, they become dark yellow, and the periphery becomes encircled by a grey and undefined 'Schwärmzone.' In the depth they appear circular, pale yellow, and mulberry-shaped.

Gelatine Tubes.—A greenish yellow round basin appears on the surface, which soon, without increasing in size, sinks into the gelatine, forming a funnel-shaped depression. The liquefaction is rapid, and a dirty yellow pellicle forms on the surface, whilst the gelatine becomes brownish yellow in colour. The cultures have a penetrating odour resembling the perspiration from feet.

Agar-agar.—Produces a pale yellow, dry, shining expansion, which later becomes of a greenish grey colour.

Glycerine-agar.—Forms a narrow but thick grey green expansion.

Blood Serum.—Produces a green white streak, at the lower end of which a drop forms. Later a delicate grey white serrated pellicle forms over the streak, at the bottom of which grey white shreds collect. Finally the serum becomes a gelatinous mass, in which the pellicle remains suspended.

Broth.—The liquid becomes turbid, and a green white deposit is formed.

Potatoes.—Thin and grey white somewhat restricted growth, which later, especially at higher temperatures, becomes covered with little bubbles of gas. The potato itself turns a greyish blue colour.

BACILLUS ARBORESCENS

LIQUEFIES GELATINE

Authority.—Percy and G. C. Frankland, *Zeitschrift für Hygiene*, vol. vi. p. 379.

Where Found.—In the rivers Thames and Lea, also in Loch Katrine water and the river Dee. Identified also by Tataroff in Dorpat water (*Die Dorpater Wasserbacterien*, 1891, p. 56); also by Tils in the Freiburg water (*Zeitschrift für Hygiene*, vol. ix. p. 312).

Microscopic Appearance.—Slender bacillus with rounded ends, about 2·5 μ long and 0·5 μ broad. Hangs together in twos and threes, but in broth cultures forms long wavy threads. No spore formation observed. Is capable of vibratory movement only. (See Plate II. 3D, 3E.)

Cultures.—

Gelatine Plates.—Under a low power is seen to form a thin axial stem, from both ends of which root-like branches extend, which gradually assume the appearance of a wheatsheaf. Slow liquefaction of the gelatine takes place, and near the colony the surface of the gelatine exhibits beautiful iridescent colours. (See Plate II. 3A, 3B, 3C.)

Gelatine Tubes.—Slowly liquefies the gelatine, producing a yellow deposit.

Agar-agar.—Produces a dirty orange-coloured pigment, and grows slowly.

Broth.—Renders the liquid turbid, and produces a yellow deposit. No pellicle forms on the surface.

Potatoes.—Produces a luxuriant and deep orange growth.

Remarks.—When introduced into the nitrate solution (see p. 27) no visible growth takes place; neither is any reduction of the nitrate to nitrite effected.

BACILLUS AËROPHILUS

LIQUEFIES GELATINE

Authority.—Liborius. Flügge, *Die Mikroorganismen*, 1886, p. 321.

Where Found.—In air. Mention of it in water by Roux, *Analyse Microbiologique de l'Eau*, Paris, 1892, p. 296. Also quoted by Lustig, *Diagnostik der Bakterien des Wassers*, 1893, p. 93.

Microscopic Appearance.—Slender bacillus with rounded ends $\frac{2}{3}$ as broad as *B. subtilis*; hangs together in twos or more, often giving rise to the appearance of threads. Forms bright oval spores when grown on agar-agar.

Cultures.—

Gelatine Plates.—Gives rise to small dots, which appear in about forty hours; under the microscope they are seen to be oval, smooth-rimmed colonies of a greenish yellow colour. The gelatine is rapidly liquefied, although the colonies remain small and unchanged.

Gelatine Tubes.—Forms a wide sack-like depression, the upper portion of which is filled with opaque liquid of a grey yellow colour, whilst beneath it is clear, containing a few isolated particles.

Potatoes.—Forms a smooth yellow expansion having a faint shining appearance resembling paraffin; later the growth becomes drier near the edge, and assumes an irregular and striped appearance.

Remarks.—It obstinately refuses to grow on any medium in the absence of air.

'RHINE WATER BACILLUS' (Burri)

LIQUEFIES GELATINE

Authority.—Burri, *Ueber einige zum Zwecke der Artcharakterisirung anzuwendende bacteriologische Untersuchungsmethoden nebst Beschreibung von zwei neuen aus Rheinwasser isolirten Bacterien*, Muenchen, 1893 (Oldenbourg).

Where Found.—In the river Rhine in the vicinity of Cologne.

Microscopic Appearance.—Bacilli about $2\frac{1}{2}$ to $3\frac{1}{2}$ μ long and about $\frac{3}{4}$ μ broad, with rounded ends; some isolated individuals are slightly bent. In broth cultures from one to two days old threads are found varying from 5 μ to 10 μ, and even reaching 90 μ in length, and from $\frac{1}{2}$ to $\frac{3}{4}$ μ broad. Under a high power these threads are apparently made up of short or longer elliptically shaped individual bacilli. No spore formation observed. In the presence of a sufficient supply of oxygen the bacilli, occurring both singly and in pairs, are capable of rotatory as well as forward movements, but the threads are motionless, as are also those bacilli taken from cultures only sparsely supplied with air. No flagella could be detected by Loeffler's method. Stains readily with the ordinary aqueous solutions of aniline colours.

Cultures.—

Gelatine Plates.— The colonies in the depth resemble dirty yellow dots. The surface colonies during the first three days are colourless and transparent, but later they assume a yellow colour, which extends in a lesser degree to the adjacent gelatine, the convex shape disappears, and they become bright yellow centres surrounded by a zone of clear liquid gelatine (on very crowded plates the whole of the gelatine is liquefied in a week). The colonies are so compact that they can be entirely removed from their fluid surroundings on the needle's point. Under the microscope the periphery of the surface colonies is wavy and the contents rough and irregular; the centre is yellowish, whilst the rim is transparent and grey, but after three days it also becomes yellow. The depth colonies are circular and smooth-rimmed, with granular contents.

Gelatine Tubes.—Produces a liquid depression wider at the surface than in the depth of the tube, and on the surface the still solid gelatine acquires a bluish grey opalescence. Yellow masses of bacteria collect at the bottom of the depression, and a few flocculent particles float on the surface, but no real pellicle is formed. In the depth of the gelatine the growth is very scanty, in consequence of the limited supply of air.

Glycerine-agar.— Produces in streaked cultures a thin, shining, honey-coloured expansion, which is dry and tough.

Potatoes.—Forms a moist, shining, thin, flat, orange or rust-coloured expansion. The colour only becomes bright orange after about six days, if the inoculation is made from a quite young broth culture.

Broth.—Renders it turbid, and produces a thick bright orange-coloured pellicle, and a yellow deposit. After fourteen days the liquid becomes almost quite clear, and on shaking the tube the pellicle breaks up into small pieces, which nearly all sink to the bottom. Microscopically the pellicle is seen to contain the short bacillar forms, but in the liquid only the long worm-like threads are found.

Milk.—The milk is only partially coagulated in the upper part of the tube, and the layer of cream floating on the surface assumes a pale yellow colour, which becomes deeper with age as a tough pellicle forms. The serum beneath the pellicle gives a faint alkaline reaction.

Remarks.—It grows well at from 15° to 20° C., but will not develop at 37° C. Broth cultures seven days old, exposed for five minutes to a boiling temperature, are destroyed, similar results being obtained when such cultures were kept for half an hour at 60° C. If the culture tube is covered with an india-rubber cap, and the access of air prevented, the bacilli very soon die. No formation of gas observed in any of the culture media, even when an addition of dextrin was made. Grows best when the culture material contains about 0·05 per cent. of carbonate of soda.

BACILLUS CONSTRICTUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Rods with rounded ends consisting of from two to six and more individual bacilli, the division between which is marked by a slight constriction. The length of the shortest is about 1·5 μ, whilst the long rods reach to 6·5 μ and more. They are about 0·75 μ broad. Rotatory and vibratory movement only. No spore formation observed.

Cultures.—

Gelatine Plates.—After from four to five days the colonies appear as small shining drops of a Naples-yellow colour. Under a low power the depth colonies are circular discs with a serrated edge of a greyish yellow colour, with granular contents. No liquefaction takes place.

Gelatine Tubes.—Forms an irregular and raised expansion of a Naples-yellow colour, and grows freely in the depth.

Agar-agar.—Produces a somewhat restricted thick and shining expansion, Naples-yellow in colour.

Potatoes.—Forms a delicate expansion of a cadmium yellow colour.

BACILLUS MUSCOÏDES

Authority.—Liborius, 'Beiträge zur Kenntniss des Sauerstoffbedürfnisses der Bacterien,' *Zeitschrift f. Hygiene*, vol. i., 1886, p. 163.

Where Found.—Isolated from mice inoculated with garden soil, old cheese, or cows' excrements. Found in water by Tils (*loc. cit.*).

Microscopic Appearance.—Bacillus about 1 μ broad; exhibits but slight inclination to form threads. Moves slowly. Forms oval-round lustrous spores at the end of the rod.

Cultures.—

Gelatine Plates.—Can only be anaërobically cultivated. Forms very delicately finely branched colonies resembling some descriptions of moss. No liquefaction takes place.

Gelatine Tubes.—Grows in the lower depths of the tube, and forms a delicately branched opalescence.

BACILLUS MULTIPEDICULOSUS

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 323.
Where Found.—In air and water.
Microscopic Appearance.—Long slender bacillus. It is not motile.
Cultures.—

Gelatine Plates.—The depth colonies are round or oval, and smooth-rimmed; at some spots on the periphery broad, radial and concentric extensions project, which consist of circular zoogloœa masses. In from two to three days these extensions are visible to the naked eye, but as they only occur here and there, and only incline to any length at one end of the colony, the general appearance is that of an insect with innumerable feet and antennae. No liquefaction takes place.

Gelatine Tubes.—Forms short isolated extensions along the needle's path in the depth.

Potatoes.—Dirty yellow, somewhat restricted, smooth expansions.

BACILLUS STOLONATUS

Authority.—Adametz-Wichmann, *Die Bakterien der Nutz- und Trink-wässer*, Adametz. Vienna, 1888.
Where Found.—In water.
Microscopic Appearance.—Bacilli two and a half times as long as broad. Very motile.
Cultures.—

Gelatine Plates.—Forms in the depth small whitish or yellowish brown colonies, round or egg-shaped, with smooth rims. On the surface, protruding ball-shaped colonies. In peptone-gelatine, without broth, they are brown in colour. No liquefaction takes place.

Gelatine Tubes.—Forms a whitish expansion at the point of inoculation, whilst in the depth a slow granular growth makes its appearance. In older cultures (about two to three weeks) the upper part of the needle's path becomes widened in the shape of a bottle or funnel, and the sides covered with white growths. No liquefaction takes place.

Agar-agar Plates.—From the centre of the colony variously twisted branches extend, from which again numerous fine irregular ramifications proceed. Under a low power it appears as a very thin, finely granular, yellowish expansion, the branches of which appear like clubs.

Potatoes.—Forms a dirty white expansion.

BACILLUS PUTRIFICUS COLI

LIQUEFIES GELATINE

Authority.—Bienstock, 'Ueber die Bakterien der Fæces,' *Zeitschrift für klin. Med.*, vol. viii.

Where Found.—In fæces. Found in water by Tils (*loc. cit.*).

Microscopic Appearance.—Slender bacillus, about 3 μ long, but often shorter, and often also giving rise to threads. Forms very lustrous spores, round in shape, which appear usually at only one end of the rod and, remaining for a time attached, recall the appearance of a drumstick. It is very motile, and the bacillus may be seen moving with the spore attached.

Cultures.—

Gelatine Plates.—The growth has at first a shining appearance of mother-of-pearl, but later it becomes yellowish. Under a low power their periphery is irregular, whilst in the centre they are coarsely granulated. The periphery appears to consist of wavy bands of bacillar threads. After some days the centre of the colony begins to sink and the gelatine becomes liquefied (Tils).

Gelatine Tubes.—Forms a delicate expansion at the point of inoculation, whilst in the depth a feeble whitish growth is visible. Later the gelatine is liquefied, and a delicate pellicle forms on the surface (Tils).

Potatoes.—Produces a dirty white or greyish restricted expansion. The potato in the vicinity of the growth becomes dark in colour (Tils).

BACILLUS PUNCTATUS

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—Frequently in the Chemnitz water supply.

Microscopic Appearance.—Medium-sized rods, consisting of from 1 to 2, rarely more, individual bacilli. The bacilli are about 0·77 μ broad, and about 1·0 to 1·60 μ long. It is very motile.

Cultures.—

Gelatine Plates.—Forms cup-shaped liquefying colonies. In the bluish grey liquid whitish dotted groups of bacteria occur, which frequently appear joined to one another by white streaks.

Gelatine Tubes.—The gelatine is liquefied in the shape of a stocking; the liquid is turbid, and a white sediment collects at the bottom. No pellicle forms on the surface.

Agar-agar.—Produces a delicate grey and shining expansion, with a uniformly smooth surface.

Potatoes.—Forms a brownish flesh-coloured expansion, which rapidly covers the whole surface, and later becomes somewhat darker.

Remarks.—It grows better at 30° C. than at the ordinary temperature.

BACTERIUM ZÜRNIANUM (List)

Authority.—Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Small bacilli slightly pointed at the ends, 0·6 to 0·8 μ broad, and 0·2 to 1·5 μ long. In stained preparations the poles stain more intensely than the middle, giving it the appearance of a diplococcus. It is not motile. Does not form spores.

Cultures.—

Gelatine Plates.—Forms circular dirty white or grey colonies composed of exceedingly viscid slime, and which gradually form grape-shaped slimy heaps. No liquefaction takes place.

Gelatine Tubes.—Forms a grape-shaped growth on the surface, but hardly grows at all in the depth. Produces a slight odour recalling that of sour cabbage.

Potatoes.—At from 25° to 30° C. it produces an extensive slimy expansion, slightly transparent, and grey or yellowish white in colour.

BACILLUS UREAE

Authority.—Jaksch, *Zeitschrift f. phys. Chemie*, vol. v. p. 395. Also Leube and Grasser, *Virchow's Archiv*, vol. c., p. 556.

Where Found.—In decomposed ammoniacal urine. Found frequently by Tils (*loc. cit.*) in the Freiburg water. Also by Lustig (*loc. cit.*) in river-water.

Microscopic Appearance.—Plump bacilli about 2 μ long and 1 μ broad, with rounded ends.

Cultures.—

Gelatine Plates.—On the second day a small, almost transparent spot is visible. The colonies resemble a glass plate which has been breathed upon. The growth extends from the centre in irregular and circular zones, the last of which has a serrated edge.

Gelatine Tubes.—After ten days thin grey extensions are visible along the needle's path in the depth, and there is but rarely much growth towards the surface. No liquefaction takes place. The growth emits a characteristic odour recalling that of herring brine.

Remarks.—It converts urea into ammonium carbonate more energetically than the *M. ureae*. (See p. 504.)

BACILLUS THERMOPHILUS

Authority.—Miquel, 'Monographie d'un Bacille vivant au-delà de 70° C.,' *Annales de Micrographie spécialement consacrées à la Bactériologie, aux Protophytes et aux Protozoaires*, Paris, No. 1, 1888, p. 3.

Where Found.—Occasionally in air. Found frequently in river-water, but not in spring-water. Very prevalent in sewage-polluted water. Miquel found as many as 1,000 in 1 c.c. of river Seine water collected at the bridge of Austerlitz in Paris, and many more in the river-water below Paris. Its normal habitat appears to be drain-water. It is also found in soil, as well as in the alimentary canal of man and animals. If drops of drain-water be inoculated into broth-tubes subsequently maintained at about 69° C., they become turbid in twenty-four hours, and in nearly all cases contain the *B. thermophilus*.

Microscopic Appearance.—The dimensions of the bacillus vary according to the temperature at which it is cultivated. Thus at 50° C. it is usually short, and exhibits an oval spore at the end of the rod. At 60° C. it forms filaments, and only a few spores are visible. At 70° C. the filaments acquire a granulated appearance. At 71° to 72° C. no spores are found, and the bacillus is swollen and resembles a necklace. It is not motile.

Cultures.—

Agar-agar Tubes.—It will neither grow on gelatine at from 22° to 23° C., nor on agar-agar between 30° and 40° C. At 42° to 45° C. a white raised meniscus-shaped growth is visible on the agar. Microscopic examination reveals the presence of a short plump bacillus, with a very highly refracting spore at one end.

Broth.—No growth at 40° C., even when preserved at this temperature for thirty days, but at 42° C. and beyond the liquid becomes turbid in three to four days, and at 50° C. in forty-eight hours, and still earlier at 60° C., when cloudy isolated patches appear in the broth. It grows best at from 65° to 70°. At 71° C. the appearance of turbidity only commences after two days, whilst at 72° C. it grows very badly. At the most favourable temperatures an abundant precipitate forms at the bottom of the tube, and the liquid becomes clear.

Remarks.—It is not pathogenic to animals. When the temperature of the water is raised beyond 50° C. it becomes an active putrefying agent. The fact that it is found in the alimentary canal indicates, says Miquel, that it is capable of growing slightly between 37° and 40° C.

'SEIDENGLÄNZENDER BACILLUS'

Authority.—Tataroff, *Die Dorpater Wasserbacterien*, Dorpat, 1891, p. 26.

Where Found.—In Dorpat well-water. Resembles very closely the *B. candicans* obtained from soil and described by Percy and G. C. Frankland (*loc. cit.*).

Microscopic Appearance.—Ovoid-shaped bacilli about 0·8 μ broad and 1·7 μ long, with rounded ends. Grows also in pairs and forms short threads. Slight oscillatory movements.

Cultures.—

Gelatine Plates.—The depth colonies are small round white lustrous balls. The surface colonies are button-shaped, milk-white, with a jagged edge, and lustrous. Under a low power the depth colonies are circular, brownish yellow, smooth-rimmed and granular. The surface colonies are brownish grey, granular, opaque in centre, but lighter towards the edge, which is lobular and radially striped. No liquefaction takes place.

Gelatine Tubes.—Forms a sword-like growth in the depth, and on the surface a small white shining radially striped jagged basin-shaped expansion. Later it becomes smooth and concave.

Agar-agar.—Forms a rather thick white skin-like expansion, which has a violet iridescent mother-of-pearl appearance. Later it becomes thinner and fatty, although the iridescence is retained. In the condensed water a rather considerable sediment collects. In glycerine-agar the skin is wrinkled, and when it becomes flatter assumes a silk-like shining appearance.

Blood Serum.—Forms here and there white wart-like growths.

Potatoes.—Forms a restricted dirty white delicate shining and smooth expansion, which later becomes light brown. In the incubator the surface becomes wrinkled, but later smoother, with the characteristic silk-like shining appearance.

Broth.—Renders the liquid turbid and forms a considerable deposit. A delicate iridescent skin forms on the surface, which sinks to the bottom, whilst a new one forms on the surface.

MICROCOCCUS BISKRA

LIQUEFIES GELATINE

Authority.—Heydenreich, *Pendinskaia Jasna*, Petersburg, 1888.

Where Found.—In water and air. Found in pus and serous exudations.

Microscopic Appearance.—Diplococcus, with a capsule; as they sometimes lie side by side in pairs, they recall the appearance of sarcina. In length they are from 0·86 to 2 μ. Not motile.

Cultures.—

Gelatine Tubes.—After forty-eight hours at from 20° to 21° C. a grey white mass appears all along the needle's path in the depth, which also at times consists of dot-shaped colonies. On the surface a circular, whitish yellow expansion forms. After from three to four days liquefaction ensues in the shape of a funnel, which becomes wider, so that in fourteen days the whole of the gelatine is fluid.

Agar-agar.—At 37° C. a greyish or yellowish white expansion is visible in twenty-four hours. The colour, however, is not constant, neither is the degree of virulence of the cultivation. The growth has a shining appearance resembling that of sealing-wax.

Potatoes.—At from 30° to 35° C. a white or yellow growth is visible on the second day. On this medium involution forms are found frequently.

Remarks.—It grows best at about 30° C., and only very slowly at 15° C. It will not grow at 45° C., and cultures exposed to 60° C. for a quarter of an hour and to 100° C. for five minutes are destroyed. It is pathogenic to rabbits, dogs, fowls, horses, and sheep. By rubbing in some of the cultures, swellings and ulcers have been produced in man.

COCCUS B. (Foutin)

Authority.—Foutin, 'Bakteriologische Untersuchungen von Hagel,' *Centralblatt f. Bakteriologie*, vol. vii., 1890, p. 372.

Where Found.—In hail.

Microscopic Appearance.—Large round coccus (about 1 μ), occurs generally in twos, threes, or short chains. Is easily coloured by Gram's method, as well as by the ordinary stains.

Cultures.—

Gelatine Plates.—The colonies on the sixth day are circular, about 1 mm. in size, white and slightly raised. Under a low power they are seen to be grey, yellowish green discs, which are slightly granular towards the periphery; sometimes the latter is smooth, and sometimes lobular. No liquefaction takes place.

Gelatine Tubes.—Forms a flat-headed, nail-like growth on the surface, whilst later on lateral branchings extend from the needle's path in the depth, resembling the growth of the *B. murisepticus* (see p. 428).

Agar-agar.—Produces a smooth-rimmed, shining, white expansion.

Potatoes.—Grows slowly, producing a thin, almost transparent, whitish expansion.

Remarks.—It is pathogenic to white rats when injected into the abdominal cavity, the cocci being found in the blood, liver, and spleen, and the animal dying in from five to six hours.

COCCUS A. (Foutin)

LIQUEFIES GELATINE

Authority.—Foutin, 'Bakteriologische Untersuchungen von Hagel,' *Centralblatt f. Bakteriologie*, vol. vii., 1890, p. 372.

Where Found.—In hail.

Microscopic Appearance.—Round cocci, which are easily stained, and also by Gram's method.

Cultures.—

Gelatine Plates.—On the fourth day slightly prominent and white discs are visible. Under a low power they are seen to be dark in the centre, the periphery being lighter and slightly granular.

Gelatine Tubes.—Forms a yellowish nail-head growth on the surface, but very little is visible in the depth. Liquefaction begins on the fifth to sixth day, and proceeds very slowly, whilst a deposit collects at the bottom.

Agar-agar.—Produces a shining, smooth, pale, rose-coloured expansion, with a sharply-defined periphery.

Potatoes.—Resembles the growth of the typhoid bacillus (see p. 410).

Remarks.—It is not pathogenic to mice and guinea-pigs.

'RHINE WATER MICROCOCCUS' (Burri)

LIQUEFIES GELATINE

Authority.—Burri, *Ueber einige zum Zwecke der Artcharakterisirung anzuwendende bacteriologische Untersuchungsmethoden nebst Beschreibung von zwei neuen aus Rheinwasser isolirten Bacterien*, Muenchen, 1893 (Oldenbourg).

Where Found.—In the River Rhine in the vicinity of Cologne.

Microscopic Appearance.—Cocci varying in diameter from $\frac{1}{2}$ to $1\frac{1}{4}$ μ, the majority, however, measure $\frac{3}{4}$ to 1 μ. Hardly ever quite round, but mostly flattened. Occurs in tetrads occasionally; no real chains are formed; three individuals, however, sometimes hang together. Not motile. Is readily stained by the ordinary aqueous solutions of aniline colours.

Cultures.—

Gelatine Plates.—The surface colonies are visible in from two to three days as minute white dots, which under a low power are seen to be circular discs with a slightly irregular edge, which is distinctly granular. After seven days the colonies are still very small, and begin to sink in the softened gelatine. Some weeks later each colony rests in a depression filled with stringy, liquid gelatine. The depth colonies, under a low power, are mostly lenticular in shape, and exhibit granular structure near the periphery.

Gelatine Tubes.—After two days a slight depression is visible at the point of inoculation which later resembles an air-bubble. At the end of eight days a long but very narrow funnel is formed containing quite clear liquid in the upper part, whilst at the bottom a white granular deposit collects, and a pellicle forms on the surface. The growth is very slow; later numerous fine flocculent particles pervade the otherwise clear liquid.

Glycerine-agar Plates.—At 30° C. after about two days pure white, soft, very shining, slightly convex discs make their appearance. Under a low power the rim is distinctly granular, but with a higher power the whole of the contents are seen to be granular; towards the periphery the granulation becomes less and less compact, and gradually becomes disintegrated. The contents are viscid. The depth colonies resemble those found on gelatine plates.

Glycerine-agar Tubes.—At 30° or 37° C. after twenty-four hours a white, shining, smeary, viscid, and irregular expansion appears all along the streak of the needle. The growth does not later increase very materially in extent.

Potatoes.—On slightly acid potatoes it produces a flat, white, and restricted expansion, which later becomes rather thicker, but remains flat. The growth is viscid and stringy. On potatoes treated with 0·05 per cent. of carbonate of soda it only grows very feebly.

Broth.—At 30° C. in twenty-four hours the liquid is very turbid, and never subsequently becomes clear. A finely granular white deposit collects at the bottom.

Sterilised Milk.—No appreciable growth and no coagulation. After fourteen days the milk has a slightly acid reaction.

Remarks.—Grows very slowly at from 20° to 22° C., but develops rapidly at from 30° to 37° C. Vigorous broth-cultures can resist half an hour's exposure to 60° C., but they are destroyed at 80° C., also when boiled once and for a short time. Develops best on neutral or slightly acid media. An addition of 0·3 per cent. of carbonate of soda to the culture material entirely stops its growth. No gas is produced even in media containing dextrin. Not pathogenic to guinea-pigs.

'GREY COCCUS'

LIQUEFIES GELATINE

Authority.—Maschek, *Jahresbericht der Oberrealschule zu Leitmeritz*, 1887.
Where Found.—In water.
Microscopic Appearance.—Occurs as diplococci and in chains. It is not motile.
Cultures.—
Gelatine Plates.—Colonies only appear on the surface. They are irregular and have a dark centre. Under a low power the centre appears to be surrounded by a woven network green in colour. The gelatine is liquefied in about six days and an odour of bad eggs is emitted.
Gelatine Tubes.—Grows only on the surface and rapidly liquefies the gelatine.
Potatoes.—Forms a bluish grey expansion. After about five weeks a smell of bad eggs is noticeable.

MICROCOCCUS CANDICANS

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 173; also Percy Frankland, 'Studies on some new Microorganisms obtained from Air,' *Phil. Trans.*, 1887, vol clxxviii. p. 270.
Where Found.—In air and water.
Microscopic Appearance.—Cocci of irregular size, the larger ones being 1 μ in diameter. They appear gathered together in groups, but exhibit no characteristic arrangement. Not motile.
Cultures.—
Gelatine Plates.—The colonies are milk-white, and under a low power they are seen to have a smooth edge, whilst the interior is granular. The older colonies are somewhat irregular in shape; the less developed ones are, however, nearly circular.
Gelatine Tubes.—After four days there is a surface depression containing an intensely white and opaque mass. As the cultivation becomes older liquefaction slowly proceeds downwards, the liquid being highly glutinous and turbid. The mode of liquefaction varies according to the temperature: in warm weather or at 22° C. it takes place in a long narrow funnel, whilst at a low temperature it is mostly confined to the surface. (Percy Frankland.) Flügge does not appear to have noted its liquefying property.
Agar-agar.—Produces in three days a vigorous growth forming a smooth and dazzling white mass, resembling a moist patch of Chinese white. (Percy Frankland.)
Potatoes.—Forms a rapidly spreading, white, slimy expansion.
Broth.—Renders the liquid turbid and produces a white deposit. No pellicle is formed.

MICROCOCCUS CANDIDUS

Authority.—Cohn, *Beiträge zur Biologie der Pflanzen*, vol. i., Heft. ii., 1870, p. 160.

Where Found.—Included by Lustig amongst bacteria found in water.

Microscopic Appearance.—Small round refracting cocci from 0·5 to 0·7 μ in diameter. Forms zoogloea. It is not motile.

Cultures.—

Gelatine Plates.—Forms at first small circular white heaps, which later become irregular in shape, having a convex surface. Under a low power the contents appear granular.

Gelatine Tubes.—Grows chiefly on the surface and is hardly visible in the depth. No liquefaction takes place.

Agar-agar.—Resembles the growth in gelatine tubes.

Remarks.—It grows in solutions of sugar, but excites no fermentation (Adametz).

MICROCOCCUS CEREUS ALBUS

Authority.—Passet, 'Ueber Mikroorganismen der eitrigen Zellgewebsentzündung des Menschen,' *Fortschritte d. Medicin*, 1885, No. 2.

Where Found.—In pus by Passet, and by Tils (*loc. cit.*) in water. It occurs also in the air.

Microscopic Appearance.—Large cocci 1·16 μ in diameter; occurs singly, but also in groups, and occasionally in chains. It is not motile.

Cultures.—

Gelatine Plates.—Forms white dots which extend on the surface to from 1 to 2 mm. They resemble drops of stearin or white wax, being faintly shining.

Gelatine Tubes.—Grows slowly in the depth, forming a grey white streak, and produces on the surface a white waxlike expansion with a thickened irregular periphery. No liquefaction ensues.

Agar-agar.—Forms a fairly thick grey white faintly shining expansion.

Potatoes.—Forms a fairly thick grey white expansion.

Blood Serum.—Forms a greyish white faintly shining streak in the depth.

MICROCOCCUS AËROGENES

LIQUEFIES GELATINE

Authority.—Miller, *Deutsche medicinische Wochenschrift*; 1886, No. 8.

Where Found.—In the alimentary canal. In water by Tils (*loc. cit.*).

Microscopic Appearance.—Large oval cocci. Not motile.

Cultures.—

Gelatine Plates.—Forms chiefly circular colonies of a grey white colour, the periphery of which is somewhat lobular, but smooth. Characteristic marbled spots are visible, which look light or dark according to the position of the microscope.

Gelatine Tubes.—Forms a flat, grey white, button-shaped growth on the surface, whilst all along the needle's track in the depth a brownish yellow growth appears. After a time slight liquefaction commences.

Agar-agar.—Produces a yellowish white mash-like expansion.

Potatoes.—Gives rise to a slimy, grey white expansion, with an irregular and lobular periphery.

Remarks.—It is very resistant to the action of acids, and will maintain its vitality for hours in artificially prepared gastric juice. In substances containing carbohydrate it produces a considerable amount of gas.

MICROCOCCUS PLUMOSUS

Authority.—Bräutigam, *Die Bakterien der Nutz- und Trinkwässer*, Adametz. Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Round cocci 0·8 μ in diameter, forming zoogloœa. Not motile.

Cultures.—

Gelatine Plates.—Forms tongue-shaped yellowish white colonies, the edges of which are generally raised like a rampart above the surface of the gelatine. No liquefaction takes place.

Gelatine Tubes.—Forms on the surface a slimy expansion, from which ramify delicate white extensions, resembling crystal needles. In the depth similar extensions are visible along the needle's path; these ramifications consist of beaded strings of colonies.

Potatoes.—Forms a yellowish white irregular expansion, with tongue-shaped projections.

MICROCOCCUS AQUATILIS

Authority.—Bolton, 'Ueber das Verhalten verschiedener Bacterienarten im Trinkwasser,' *Zeitschrift f. Hygiene*, vol. i., 1886, p. 94.

Where Found.—Very frequently in water.

Microscopic Appearance.—Very small cocci, gathered together in groups.

Cultures.—

Gelatine Plates.—Forms circular, porcelain-white, smooth, and slightly raised colonies. Under a low power the depth colonies are roundish, with a rough denticulated edge, and resemble a mulberry in shape, and are of a light yellow colour. The surface colonies are circular and smooth-rimmed; the centre is dark, and numerous furrows extend from it, enclosing small rhombic irregular spaces. No liquefaction takes place.

Gelatine Tubes.—Grows on the surface and along the needle's path in the depth, producing a white growth.

Agar-agar.—Forms a white expansion.

PEDIOCOCCUS ALBUS

LIQUEFIES GELATINE

Authority.—Lindner, *Die Sarcine-organismen der Gärungsgewerbe*, Berlin, 1888.

Where Found.—In well-water.

Microscopic Appearance.—Cocci arranged as diplococci and in tetrads. It does not exhibit the typical sarcina form, but occurs frequently in a pseudo-sarcina form, when the tetrads lying close together become pushed one on the top of the other.

Cultures.—

Gelatine Plates.—Rapid liquefaction of the gelatine takes place, the ball-shaped colony sinking to the bottom and forming later an irregular flocculent mass.

Gelatine Tubes.—In twenty-four hours the whole length of the needle's path in the depth is liquefied, and at the bottom of the canal a white flocculent sediment collects, which on the fourth day assumes a faint orange tone.

Agar-agar.—Forms a broad and dry expansion, which later becomes orange-coloured.

Potatoes.—Forms a dirty white cake-like expansion.

Remarks.—In culture fluids it rapidly forms a pellicle. It grows best at 20° to 25° C., but will also develop at 40° C. It will withstand an exposure of twelve minutes to 50° to 55° C., but an exposure to 60° C. for eight minutes destroys it.

MICROCOCCUS VIOLACEUS

Authority.— Schroeter, 'Ueber einige durch Bacterien gebildete Pigmente,' *Cohn's Beiträge zur Biologie der Pflanzen*, vol. i., Heft ii., p. 124.

Where Found.—In air and water.

Microscopic Appearance.—Elliptically shaped cocci, larger than those of *B. prodigiosus*, and arranged in chains. Not motile.

Cultures.—

Gelatine Plates.—Forms slimy drop-like raised growths of a violet colour; the latter appear on the surface of the colony. No liquefaction ensues.

Potatoes.—Originally obtained from air by Schroeter on exposed slices of potato, appearing as slimy spots of a strong violet blue colour, which gradually coalesced and produced a flat violet-coloured expansion. (Schroeter, *loc. cit.*)

MICROCOCCUS CYANEUS

Authority.—Schroeter, 'Ueber einige durch Bacterien gebildete Pigmente,' *Beiträge z. Biologie der Pflanzen*, vol. i., Heft ii., 1870, p. 122; also Cohn, 'Untersuchungen über Bacterien,' *loc. cit.*, p. 156.

Where Found.—In air by Schroeter. Included by Lustig amongst bacteria found in water.

Microscopic Appearance.—Elliptical cocci. Not motile. Forms zoogloea.

Cultures.—

Gelatine Plates.—Forms small, circular, well defined colonies. Under a low power the centre is bluish in colour, and is surrounded by an irregular network.

Gelatine Tubes.—It will not grow in the depth, but forms a slimy mass on the surface. No liquefaction ensues.

Potatoes.—Develops slowly (about ten days), forming a dark indigo blue growth which penetrates into the depth of the potato. When examined under the microscope no bacteria were found in the depth. (Schroeter.)

Remarks.—The pigment resembles litmus in colour.

MICROCOCCUS CONCENTRICUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Cocci 0·9 μ in diameter, arranged in irregular groups. Not motile.

Cultures.—

Gelatine Plates.—The depth colonies are small bluish grey dots. On the surface it forms at first small bluish grey discs, which gradually become larger and more irregular. After five days the centre is greyish white, and is surrounded by a bluish grey irregular and lobular periphery. Under a low power the depth colonies are light brownish or greenish yellow granular circular discs, in the interior of which several almost regular, concentric circles are visible. The surface colonies exhibit in the interior a greyish brown dark disc, with an irregular and lobular edge, with fine radial serrations in places; round this a lighter brown granular ring, likewise with an irregular and lobular edge, is visible; the whole is enclosed by a whitish and shining border. No liquefaction takes place.

Gelatine Tubes.—Forms a thin bluish grey or whitish expansion which exhibits concentric circles, starting from the point of inoculation.

Agar-agar.—Produces a broad flat bluish grey white shining expansion, with a serrated edge.

Potatoes.—Forms a thin yellowish grey smeary growth.

MICROCOCCUS CINNABAREUS

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 174.
Where Found.—In air and water.
Microscopic Appearance.—Large round cocci; occurs often as diplococci, but each half is perfectly round; it often hangs together in threes and fours.
Cultures.—
Gelatine Plates.—It grows very slowly, the colonies in the depth after four days are only just visible as dots. In about eight days the surface colonies are raised like buttons above the surface of the gelatine. They are at first of a bright sealing-wax-red colour, but later become vermilion. Under a low power the smallest colonies in the depth are lentil-shaped, smooth-rimmed, and of a dark brown red colour. The surface colonies are light brown, transparent at the periphery, circular, but with some irregularities in the periphery. No liquefaction takes place.
Gelatine Tubes.—After from four to five days isolated white colonies are visible in the depth, whilst a moderately-sized button-shaped growth forms on the surface, at first pink but later vermilion in colour.
Potatoes.—Grows very slowly, producing a vermilion expansion.

MICROCOCCUS CARNEUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.
Where Found.—In the Chemnitz water supply.
Microscopic Appearance.—Medium-sized coccus arranged in groups like grapes. The individual cells are on an average 0·83 μ. It is not motile.
Cultures.—
Gelatine Plates.—The colonies in the depth are small grey white centres; on the surface they are greyish or pale red, slightly raised and circular expansions. Under a low power the rim is at first smooth and the colony is of a uniform greyish red colour. Later the centre becomes darker and is surrounded by a lighter circular zone, which is again encircled by a still lighter zone. In older cultures these bands are not so distinctly marked. No liquefaction takes place.
Gelatine Tubes.—Forms a thin, irregular, circular pale red expansion, which is only slightly raised above the surface of the gelatine. In the depth the needle track is marked by a fine white granular growth.
Agar-agar.—Forms a deep flesh-coloured expansion with a play of violet. The edge is lobular and serrated.
Potatoes.—Grows abundantly, producing a fine red-lead coloured expansion, which is shining at first, but becomes dull later.

Remarks.—It grows best at 20° to 22° C., and only very scantily at 30° to 33° C.

MICROCOCCUS AGILIS

LIQUEFIES GELATINE

Authority.—Ali-Cohen, 'Eigenbewegung bei Mikrokokken,' *Centralblatt f. Bakteriologie*, vol. vi., 1889, p. 33.

Where Found.—In drinking water.

Microscopic Appearance.—Occurs principally as diplococci; forms also short streptococci, and is found sometimes in tetrads. The cocci are 1 μ in diameter, and are motile. Loeffler (see p. 57) stained the flagella for the first time, and found them to be very long and fine, their length exceeding the diameter of the coccus four to five times. In older cultures the cocci lose their motility, but if inoculated into 5 per cent. milk-sugar agar they at once regain their power of movement. This was found to be the case with even three-months-old cultures.

Cultures. –

Gelatine Tubes.—Produces a pinkish red pigment, and liquefies the gelatine very slowly; for some time the needle's track in the depth only exhibits a dry, hollow funnel, and liquefaction only really commences after from three to four weeks.

Agar-agar.—Produces a pinkish red pigment.

Potatoes.—Produces a pinkish red pigment.

Remarks.—It will not grow at 37° C.

MICROCOCCUS CERASINUS SICCUS (List)

Authority.— List, *Die Bakterien der Nutz- und Trinkwässer*, Adametz. Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Very small cocci, from 0·25 to 0·32 μ in diameter. Occurs singly, and also arranged as diplococci. Not motile.

Cultures.—

Agar-agar.—Forms a dry dull expansion, which rapidly spreads and is of a cherry-red colour. It does not grow in the depth.

Potatoes.—Develops rapidly at 37° C., forming a dry red growth which quickly spreads over the whole surface.

Remarks.—It grows best at 37·5° C. The pigment produced is insoluble in water, alcohol, and ether, and is not affected in the presence of acids and alkalies. Has no fermentative properties.

MICROCOCCUS FUSCUS

LIQUEFIES GELATINE

Authority.—Maschek, *Die Bakterien der Nutz- und Trinkwässer*, Adametz. Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Cocci often elliptical in shape and sometimes difficult to distinguish from short bacilli. They are sometimes arranged in torula form. Not motile.

Cultures.—

Gelatine Plates.—Forms circular colonies, which under a low power appear light brown or blackish in colour. The interior of the colony consists of fine crevasses. Liquefaction begins early, and a dark brown pigment is produced.

Gelatine Tubes.—Hardly any growth appears in the depth; on the surface a sepia brown pellicle is formed, and the liquid gelatine emits a most penetrating and foul odour.

Potatoes.—Forms a slimy, brown expansion, which becomes darker and darker in colour.

STAPHYLOCOCCUS PYOGENES AUREUS

LIQUEFIES GELATINE

Authority.—Rosenbach, *Mikroorganismen bei den Wundinfectionskrankheiten des Menschen*, Wiesbaden, 1884; also Passet, 'Ueber Mikroorganismen der eitrigen Zellgewebsentzündung des Menschen,' *Fortschritte d. Med.*, 1885, No. 2. See also A. E. Fick, *Ueber Mikroorganismen im Conjunctival Sack*, Wiesbaden, 1887.

Where Found.—Very frequently in pus; also in air, soil and water. Found in the conjunctival sac of the eye by A. E. Fick (*loc. cit.*).

Microscopic Appearance.—Cocci of variable size arranged in heaps, and also as diplococci, or in very short chains of three or four individuals. The mean diameter of a single coccus is about 0·87 μ. It is not motile. Is readily coloured by Gram's method.

Cultures.—

Gelatine Plates.—On the second day orange dot-shaped colonies are visible, surrounded by a slight smooth-rimmed depression.

Gelatine Tubes.—A cloudy grey streak first appears, which after three days liquefies the gelatine, producing a yellow, but later an orange-coloured growth, which sinks to the bottom.

Agar-agar.—Forms a yellow and then orange-coloured expansion, with a wavy periphery.

Potatoes.—Produces a thin whitish expansion which gradually becomes moister and orange yellow in colour. Emits a strong odour of paste.

Blood Serum.—Grows as on agar-agar.

Remarks.—Grows best at 30° to 37° C. It can exist for a long time in the absence of air (Rosenbach). It is pathogenic to animals according to the manner in which it is introduced into the system. When injected into the abdominal cavity, mice, guinea-pigs, and rabbits succumb after a few days. When subcutaneously introduced into guinea-pigs and rabbits, abscesses are formed. When inoculated into the cornea, it causes acute inflammation, which lasts generally for three weeks (A. E. Fick).

MICROCOCCUS CITREUS (List)

Authority.— Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Very large and perfectly round cocci from 1·5 to 2·2 μ in diameter. Occurs singly, also arranged as diplococci, or in chains of 8 or more cocci. Not motile.

Cultures.—

Gelatine Plates.—Forms dirty yellow cream-coloured colonies which project above the level of the gelatine. They are moist and shining, and usually of irregular contour. No liquefaction takes place.

Gelatine Tubes.—Grows very slightly in the path of the needle in the depth.

Agar-agar.—Forms a light yellow expansion.

Potatoes.—Forms an abundant yellow growth at 37·5° C.

MICROCOCCUS LUTEUS

Authority.—Schroeter, *Beiträge zur Biologie der Pflanzen*, vol. i., Heft ii., 1870; also Cohn, *loc. cit.*, p. 153. See also Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In air and water.

Microscopic Appearance.—Elliptically shaped cocci, 1 to 1·2 μ in diameter. Forms zoogloœa, the intercellular substance of which is easily soluble in water. Not motile.

Cultures.—

Gelatine Plates.—Forms raised sulphur-yellow colonies, with an irregular contour. Under a low power they are slightly granular. No liquefaction takes place.

Gelatine Tubes.—Forms a yellow expansion on the surface, which later becomes wrinkled, whilst along the needle's path in the depth a granulated growth is visible.

Agar-agar.—Produces a slimy yellow expansion.

Potatoes.—Forms an intensely yellow expansion, irregular in contour, and which later becomes wrinkled.

Remarks.—The pigment is insoluble in water, alcohol, and ether.

MICROCOCCUS CREMOÏDES

LIQUEFIES GELATINE

Authority.—Zimmermann, *Die Bakterien unserer Nutz- und Trinkwässer, insbesondere des Wassers der Chemnitzer Wasserleitung*, Chemnitz, 1890.

Where Found.—In the Chemnitz water supply.

Microscopic Appearance.—Cocci about 0·8 μ in diameter, grouped together like a bunch of grapes. Not motile.

Cultures.—

Gelatine Plates.—In the depth small yellowish white dots are visible. Under a low power they are circular discs, smooth-rimmed, and with yellowish or greyish brown granular contents. On reaching the surface the contour becomes lobular and denticulated, and the gelatine becomes liquid in a saucer-like depression. The yellowish white colony covers the bottom of the depression, and becomes arranged in concentric rings. Under a low power such colonies consist of brownish yellow granular aggregates, surrounded by a less dense granular circle, which is enclosed by a transparent liquid zone in which granular particles are scattered here and there. From the periphery delicate and radial extensions often stretch into the adjacent gelatine.

Gelatine Tubes.—In from three to four days the path of the needle in the depth is liquefied, whilst on the surface a bubble of gas is usually present, beneath which yellowish white masses of bacterial growth collect, and lower down the liquid is clear or contains but a few granular particles, whilst the bottom of the funnel again is filled with the yellowish white growths. After from six to seven days a yellowish white pellicle floats on the surface of the liquid gelatine.

Agar-agar.—Forms a yellowish white amber-like shining expansion, which is irregular and lobular at the periphery, and granulated on the surface.

Potatoes.—Grows abundantly, producing a raised cream-coloured expansion.

Remarks.—It will not grow in the absence of oxygen.

MICROCOCCUS FERVIDOSUS

Authority.—Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Small round cocci 0·6 μ in diameter; occurs as diplococci, also in small groups. Not motile.

Cultures.—

Gelatine Plates.—The depth colonies appear as small white dots after from four to five days. Under a low power they are faint yellow in colour, highly refracting, smooth-rimmed, and resemble drops of dew. The surface colonies, after from five to six days, are transparent and yellow, with a serrated edge and numerous indentations and lobular projections. In older colonies the centre is granular and brownish in colour, whilst the yellowish growth surrounding it exhibits a slight folded appearance. No liquefaction takes place.

Gelatine Tubes.—Forms a very thin surface expansion, circular, and finely serrated, whilst a granular growth appears in the depth.

Glycerine-gelatine.—Gives rise to numerous bubbles of gas all along the needle's path in the depth.

Agar-agar.—Forms a circular milk-white slimy expansion which exhibits later a mother-of-pearl iridescence.

Potatoes.—Produces a dirty white irregular expansion.

Remarks.—In cane, grape, and milk-sugar solutions respectively it develops lowly, causing a slight turbidity (Tils).

MICROCOCCUS AURANTIACUS

Authority.—Schroeter, *Beiträge z. Biologie der Pflanzen*, vol. i., Heft ii., 1870, p. 119; also Cohn, *loc. cit.*, p. 154. See also *Die Bakterien der Nutz- und Trinkwässer*, by Adametz, Vienna, 1888.

Where Found.—In air and water.

Microscopic Appearance.—Round or slightly oval cocci, having a diameter of from 1·3 to 1·5 μ. They occur singly or as diplococci, or gathered together in small heaps. Not motile.

Cultures.—

Gelatine Plates.—Forms round or elliptical orange yellow centres, with a smooth, shining surface. Under a low power they are seen to be finely granular. No liquefaction ensues.

Gelatine Tubes.—Grows chiefly on the surface in the form of small yellow pin-heads, whilst in the depth along the needle's path small orange yellow dots very slowly make their appearance.

Agar-agar.—Forms an orange yellow expansion.

Potatoes.—Produces a slimy, yellow growth.

Remarks.—The orange yellow pigment is insoluble in water, alcohol, and ether.

MICROCOCCUS FLAVUS LIQUEFACIENS

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 174.

Where Found.—In air and water.

Microscopic Appearance.—Rather large cocci, occurs mostly in twos or threes, or in groups. Not motile.

Cultures.—

Gelatine Plates.—After two days small yellowish centres are visible, surrounded by a flat circular depression. Under a low power the depth colonies are circular or oval, and have also sometimes a lobular projection at one point. The surface colonies are sharply defined and finely serrated, and are of a yellowish brown colour; when liquefaction has commenced the periphery forms a sharp circular liquid ring, which is broken through in places by isolated aggregates of cocci, so that later the neighbouring colonies become mixed up with one another. The yellow centre of the colony is surrounded by a broad clear liquid zone, in which isolated radial extensions from the centre are visible. At this stage the colony resembles a carriage-wheel.

Gelatine Tubes.—All along the needle's path in the depth small round yellow colonies appear, which rapidly liquefy the gelatine, so that in eight days the test-tube contains in the upper layers clear liquid, and at the bottom a yellow flocculent deposit.

Potatoes.—Produces an irregular, intensely yellow expansion.

MICROCOCCUS FLAVUS DESIDENS

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 177.
Where Found.—In air and water.
Microscopic Appearance.—Small cocci arranged chiefly as diplococci, but also in a triangular form, and in short chains. Not motile.
Cultures.—
Gelatine Plates.—In two days the colonies are visible as small white yellowish dots. Under a low power they are oval and often have a lobular projection on one side, yellowish brown in colour, and finely granular. The surface colonies have a lighter zone near the periphery. After four days the depth colonies have hardly changed, but those on the surface are from 5 to 10 mm. large, and are circular and lobular, and of a pale yellow or brownish colour. They form a smooth slimy expansion, but neither rise above the surface nor sink much below it; later, however, the colony slowly sinks, and becomes surrounded by a very flat circular depression.
Gelatine Tubes.—Produces a confluent porcelain-white growth in the depth, and a yellowish brown slimy expansion on the surface, which, however, does not reach the walls of the tube. After eight days the gelatine below is so softened that it liquefies, forming a thick fluid, the surface gradually sinking to the bottom.
Potatoes.—Forms a yellowish brown, thick and slimy expansion, with an irregular periphery.

MICROCOCCUS FLAVUS TARDIGRADUS

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 175.
Where Found.—In air and water.
Microscopic Appearance.—Large round cocci, exhibiting sometimes characteristic dark poles. It is arranged chiefly in groups.
Cultures.—
Gelatine Plates.—It grows extremely slowly. The depth colonies are dark chrome yellow, and either round or oval in shape. The surface colonies have a smooth sealing-wax-like surface, and project slightly above the level of the gelatine. Under a low power the depth colonies are circular and smooth-rimmed, and of a uniform dark olive-green colour; the surface colonies towards the periphery are lighter in colour, being more of a greyish yellow tone. No liquefaction takes place.
Gelatine Tubes.—In six to eight days a number of isolated round yellow colonies become visible along the path of the needle in the depth.

MICROCOCCUS RADIATUS

LIQUEFIES GELATINE

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 176.

Where Found.—In air and in water by Adametz, *loc. cit.*

Microscopic Appearance.—Small cocci 0·8 μ to 1·0 μ in diameter, occurring singly, in short chains, or in groups. Is slightly motile. (See Eisenberg, *loc. cit.*, p. 23.)

Cultures.—

Gelatine Plates.—Forms large white, slightly liquefying colonies in two days, with a yellowish green fluorescence. Under a low power they are brownish yellow, granular, circular, with starfish-like extensions. In two more days' time the latter develop into a delicate and regularly-arranged circlet of rays. Often a second, sometimes even a third circlet is formed.

Gelatine Tubes.—Radial extensions ramify in places from the needle's path into the adjacent gelatine, whilst at the same time a pointed, funnel-shaped, slowly liquefying canal is formed.

Potatoes.—Grows rapidly, producing a yellowish brown expansion.

MICROCOCCUS ROSETTACEUS

Authority.—Zimmermann, *Die Bakterien unserer Trink- und Nutzwässer, insbesondere des Wassers der Chemnitzer Wasserleitung;* Chemnitz, 1890.

Where Found.—In Chemnitz water.

Microscopic Appearance.—Irregularly sized round or elliptical cocci, arranged like a bunch of grapes. Their diameter varies from 0·7 μ to over 1·0 μ. Not motile.

Cultures.—

Gelatine Plates.—Forms in the depth small greyish white dots, whilst the surface colonies form somewhat broad, greyish yellow, shining, drop-like expansions, with an irregular contour. Under a low power the former are circular and smooth-rimmed, and are only rarely lentil-shaped. These lentil-shaped colonies have an irregular periphery, and are of a brownish colour, which is darker in the centre than towards the edge. No liquefaction ensues.

Gelatine Tubes.—Forms a circular, spreading, rosette-shaped expansion. Very little growth is visible in the depth.

Agar-agar.—Forms a smooth grey shining, spreading expansion, with a delicately denticulated periphery.

Potatoes.—Forms a yellowish grey expansion.

Remarks.—It will not grow in the absence of air.

MICROCOCCUS STELLATUS

Authority.—Maschek, *Jahresbericht der Oberrealschule zu Leitmeritz*, 1887.

Where Found.—In water.

Microscopic Appearance.—It does not form chains.

Cultures.—

Gelatine Plates.—Forms star-shaped colonies due to numerous extensions (6 to 15) which start from the centre, and the ends of which are branched.

Gelatine Tubes.—Grows at first only on the surface, but later growths appear in the depth, from which branchings extend into the surrounding gelatine. The latter becomes brownish yellow in colour. No liquefaction of the gelatine takes place.

Potatoes.—Produces in fifteen days a brownish yellow slimy expansion.

MICROCOCCUS UREAE (Pasteur)

Authority.—Pasteur, *Comptes rendus*, vol. l., 1860. Van Tieghem, *Compt. rend.*, vol. lviii., 1864. Jacksch, *Zeitschrift f. physiologische Chemie*, vol. v., 1881, p. 395. Leube and Grasser, *Virchow's Archiv*, vol. c., p. 556.

Where Found.—In decomposed ammoniacal urine, also in air. Found by Tils (*loc. cit.*) in water.

Microscopic Appearance.—Cocci from 0·8 to 1·0 μ in diameter; occurs frequently arranged as diplococci and in tetrads, also frequently in more or less long chains. Grows also in the shape of zoogloœa and rose-wreaths (Jacksch).

Cultures.—

Gelatine Plates.—Forms small white shining spots having a mother-of-pearl lustre, and a smooth surface and sharply rounded contour. At the ordinary temperature of a room they reach in ten days' time the size of a sixpence and are slightly raised above the level of the gelatine, and resemble drops of wax. No liquefaction takes place.

Gelatine Tubes.—Forms a thin tough thread-like growth along the needle's path in the depth. Old cultures have a smell of paste.

It grows best in Jacksch's culture-fluid: in 1 litre $\frac{1}{16}$ grm. magnesium sulphate, $\frac{1}{8}$ grm. acid potassium phosphate, 5 grms. sodium potassium tartrate, and 5 grms. urea.

Remarks.—According to Jacksch, the most favourable temperature for its growth is 30° to 33° C.; below 0° no growth takes place, but it can be kept for several days at –13° C. without being destroyed. It converts urea into ammonium carbonate.

MICROCOCCUS VERSICOLOR

Authority.—Flügge, *Die Mikroorganismen*, 1886, p. 177.

Where Found.—Frequently in air. Found also frequently in water by Tils (*loc. cit.*).

Microscopic Appearance.—Small cocci, arranged in pairs or in aggregates.

Cultures.—

Gelatine Plates.—Forms in the depth white dot-shaped centres in twenty-four hours, which in two days become yellow in colour. Under a low power these are circular, smooth-rimmed, finely granular, and of an opaque yellowish green colour. The surface colonies form large expansions, irregular in shape, often four-cornered, with lobular projections. They are slimy, shining, and yellowish green in colour, but in some lights they have a greenish or bluish mother-of-pearl lustre. The centre of the colony is often raised. No liquefaction takes place.

Gelatine Tubes.—Forms a mother-of-pearl expansion with an irregular and frayed edge. In the depth small ball-shaped, yellow colonies make their appearance.

MICROCOCCUS VITICULOSUS

Authority.—Katz and Flügge, *Die Mikroorganismen*, 1886, p. 178.

Where Found.—Occasionally in air and water.

Microscopic Appearance.—Oval cocci of variable dimensions, the largest being about 1·2 μ in diameter, and the smaller ones 1 μ. Forms closely-packed masses of zoogloœa.

Cultures.—

Gelatine Plates.—The depth colonies exhibit fine hairy ramifications, which extend from the centre of the colony to some distance into the surrounding gelatine and form a very fine and delicate network. Under a low power these ramifications are seen to consist of zoogloœa masses of various dimensions and of irregular contour, arranged side by side. The surface colonies form a thin cloud-like expansion, opaque and whitish, from which fine threads penetrate into the depth of the gelatine. No liquefaction ensues.

Gelatine Tubes.—A delicate network appears in the depth, which soon becomes covered up by the more rapidly growing surface expansion. Along the path of the needle in the depth radial extensions resembling a feather make their appearance.

Potatoes.—Forms a dirty white dry expansion, which grows quickly.

SARCINA ALBA

LIQUEFIES GELATINE

Where Found.—In air and water.

Microscopic Appearance.—Small cocci arranged in twos or fours.

Cultures.—

Gelatine Plates.—Grows slowly, giving rise to small round white colonies. Slight liquefaction slowly ensues.

Gelatine Tubes.—Grows only slightly in the depth, but produces a white raised circular growth on the surface.

Potatoes.—Grows very slowly, producing a whitish yellow expansion, restricted to the point of inoculation.

SARCINA CANDIDA (Reinke)

LIQUEFIES GELATINE

Authority.—Lindner, *Die Sarcine-organismen der Gärungsgewerbe*, Berlin, 1888.

Where Found.—In a water reservoir supplying a brewery; also in air in the vicinity of breweries.

Microscopic Appearance.—Occurs as cocci, or diplococci and tetrads. The individual cells have a diameter of 1·5 to 1·7 μ. They are only arranged in the three dimensions of space, in particular culture media (decoction of hay).

Cultures.—

Gelatine Plates.—Forms shining white colonies surrounded by a circle of liquid gelatine. Later they become yellowish in colour.

Gelatine Tubes.—Resembles the growth of the *Pediococcus albus* (see p. 494).

Agar-agar.—Forms a white, moist, shining expansion with a smooth rim. No pellicle forms on the condensed water.

SARCINA LUTEA

LIQUEFIES GELATINE

Authority.—Schroeter, 'Ueber einige durch Bacterien gebildete Pigmente, *Beiträge z. Biologie der Pflanzen*, vol. i., Heft ii., in note at foot of p. 119; also 'Studies on some new Micro-organisms obtained from Air,' Percy Frankland, *Phil. Trans. Roy. Soc.*, vol. clxxviii., 1887, p. 265.

Where Found.—Originally by Schroeter on potatoes exposed to the air. Found also in water by Tils (*loc. cit.*). Found in the conjunctival sac of the eye by A. E. Fick, *Ueber Microorganismen im Conjunctival Sack*, Wiesbaden, 1887, p. 48.

Microscopic Appearance.—Very large cocci from 1·5 to 2·5 μ in diameter, and arranged in twos and fours, and in the three dimensions of space. They stain easily with very weak solutions of methyl violet, and are not decolourised when subsequently treated by Gram's method (Fick). The arrangement in cubical packets is especially well seen in drop-cultures. It is not motile.

Cultures.—

Gelatine Plates.—Grows very slowly, producing small, round, yellowish colonies. Under a low power the centre of the colony is of a dark greyish green colour; it is finely granular, and lighter in colour near the periphery, and the edge is slightly irregular. (Percy Frankland.)

Gelatine Tubes.—It grows slowly, forming numerous minute yellow centres in the track of the needle, whilst on the surface it produces a shining lemon-yellow expansion consisting of small hump-like protuberances. In nine days the surface growth was still very restricted, but had formed a depression filled with lemon-yellow semi-liquid matter. Even after eighteen days there was but little change in the needle-track, but the surface-depression, which was considerable, was filled with liquid, at the bottom of which was a lemon-yellow deposit. (Percy Frankland.)

Agar-agar.—Forms a thick chrome-yellow moist mass, extending over the surface.

Potatoes.—Grows very slowly, producing sulphur-yellow colonies restricted to the point of inoculation.

Broth.—After nine days the liquid is clear and free from pellicle, whilst a lemon-yellow deposit collects at the bottom. (Percy Frankland.)

SARCINA AURANTIACA

LIQUEFIES GELATINE

Authority.—Koch. Employed by Fischer and Proskauer in their experiments, 'Ueber die Desinfection mit Chlor. Brom.,' *Mittheilungen a. d. Kaiserlichen Gesundheitsamt*, vol. ii., 1884, p. 240. See also 'Studies on some new Micro-organisms obtained from Air,' Percy Frankland, *Phil. Trans. Royal Soc.*, vol. clxxviii., 1887, p. 266.

Where Found.—In air and Berlin white beer. Included by Lustig (*loc. cit.*) amongst organisms found in water.

Microscopic Appearance.—Small hemispherical cocci arranged in twos or fours, and in packets. The packets are much smaller than those of the *Sarcina lutea* (see p. 506), and the complete packet of four measures only about 1·7 μ across. It is not motile.

Cultures.—

Gelatine Plates.—On the fifth day small, round, yellow colonies are visible, each of which exhibits a circular surface depression of varying size. Under a low power they are circular and granular, with a slightly denticulated edge, which in the less developed colonies are not so marked. (Percy Frankland.)

Gelatine Tubes.—After four days liquefaction has taken place along the path of the needle, producing a funnel-shaped canal which is filled with clear liquid, at the bottom of which is a flocculent orange deposit.

Agar-agar.—Forms an abundant and moist and shining surface-growth of a strong orange colour. The growth is for the most part continuous, but numerous little heaps are distributed over the remainder of the surface.

Potatoes.—Grows very slowly, producing a golden yellow expansion restricted to the seat of inoculation.

Broth.—After nine days the liquid is turbid at the surface, but clear below, with a dirty white deposit at the bottom. After eighteen days the deposit has become of an orange colour. (Percy Frankland.)

SARCINA LITORALIS

Authority.—Oersted.

Where Found.—In sea-water containing putrefying matter.

Microscopic Appearance.—Cocci 1·2 to 2 μ in diameter, grouped together in fours and eights, which may unite and include as many as sixty-four tetrads. Plasma colourless; in each cell one to four sulphur granules are visible.

SARCINA HYALINA

Authority.—Kützing.

Where Found.—In marshes.

Microscopic Appearance.—Round cocci, 2·5 μ in diameter. United in groups of four to twenty-four cells, reaching 15 μ in diameter. Almost colourless.

SARCINA REITENBACHII

Authority.—Caspary.

Where Found.—On rotting water plants.

Microscopic Appearance.—Cocci about 1·5 to 2·5 μ in diameter, at the time of division lengthened to 4 μ. Mostly united together from four to eight in number; occasionally sixteen or more. Colourless cell-wall, lined with rose-red layer of plasma.

STREPTOCOCCUS ALBUS

LIQUEFIES GELATINE

Authority.—Maschek, *Jahresbericht der Oberrealschule zu Leitmeritz*, 1887.
Where Found.—In water.
Microscopic Appearance.—Cocci, which are motile only during the period of division.
Cultures.—
Gelatine Plates.—Flat expansions with a white periphery. Under a low power a small dark yellow cloud is visible in the centre. Liquefaction proceeds rapidly.
Gelatine Tubes.—Forms a flat expansion, which rapidly liquefies the gelatine and produces a white deposit.
Potatoes.—Grows rapidly, producing a slimy expansion.

STREPTOCOCCUS VERMIFORMIS

LIQUEFIES GELATINE

Authority.—Maschek, *Jahresbericht der Oberrealschule zu Leitmeritz*, 1887.
Where Found.—In water.
Microscopic Appearance.—The individual cocci are almost always so arranged that they resemble filaments which have a slow vermiform movement.
Cultures.—
Gelatine Plates.—Forms yellowish white centres, which sink into the gelatine; the centre is light, whilst the periphery is composed of a dark ring. Under a low power the contents of the colony are granular, whilst the rim exhibits a radiated structure. It liquefies the gelatine very rapidly.
Potatoes.—Forms a dirty yellow expansion, which grows very quickly.

STREPTOCOCCUS MIRABILIS

Authority.—Roscoe and Lunt, 'Contributions to the Chemical Bacteriology of Sewage,' *Phil. Trans. Royal Society*, vol. clxxxii., 1892, p. 648.
Where Found.—In sewage.
Microscopic Appearance.—Streptococci, forming very long chains. The individual cocci are 0·4 μ thick, but the diplococci undergoing fission are about 1·2 μ. It is not motile.
Cultures.—
Gelatine Plates.—It grows badly; the depth colonies, even after four days, are mere microscopic dots or gnarled and convoluted thread-like masses. Some surface colonies exhibit an exceedingly faint and transparent expansion, about 2 millims. in diameter. Under a low power it is seen to consist of a mass of fine long threads, sometimes throwing out processes into the surrounding gelatine. No liquefaction takes place, and growth apparently ceases after the first five or six days.
Gelatine Tubes.—Produces an exceedingly faint and transparent film, almost invisible to the naked eye. It attains a diameter of about three to five millims. in a few days, after which the growth ceases.
Agar-agar.—Resembles the growth on gelatine.
Potatoes.—Inappreciable growth.
Broth.—In forty-eight hours nearly the maximum growth has taken place; a fine mass resembling delicate cotton-wool collects at the bottom of the tube, or is carried upwards in delicate festooned threads by the convection currents in the liquid. The threads sink, however, to the bottom when taken out of the incubator (20° to 23° C.), and the broth above remains perfectly clear.
Remarks.—It grows quite as readily in broth in an atmosphere of pure hydrogen as in air. Its power of absorbing atmospheric oxygen was investigated. After seven days it was found to be almost nil. For an account of the methods employed consult the original paper, p. 637.

DIPLOCOCCUS LUTEUS

LIQUEFIES GELATINE

Authority.—Adametz, *Die Bakterien der Nutz- und Trinkwässer*, Vienna, 1888.

Where Found.—In water.

Microscopic Appearance.—Cocci about 1·2 to 1·3 μ in diameter. It occurs mostly as diplococci, but forms also small groups and also chains of ten individuals. It is very motile; the chains have a worm-like movement.

Cultures.—

Gelatine Plates.—In three days circular, bright-yellow colonies of a tough, slimy consistency are visible. Under a low power the centre is yellowish brown; towards the rim, which is smooth, the colour is bright yellow. In six days the colony is 3 mm. in diameter, and is of an intensely yellow colour.

Gelatine Tubes.—Forms an abundant growth on the surface. The growth is circular, lemon-yellow in colour, and exhibits concentric circles. In about ten days an intensely brownish red colour appears in the gelatine beneath the surface growth, and seems to pervade it like a cloud, becoming less intense lower down. The gelatine is softened, and after some weeks liquefaction commences.

Agar-agar.—Produces a tough, slimy, yellow growth on the surface, whilst in the depth the agar becomes of a brownish red tint.

Potatoes.—Forms a dirty yellow expansion, which later becomes brownish and emits an odour characteristic of moulds.

Milk.—In five days the milk is coagulated.

Remarks.—Does not ferment sugar solutions.

BEGGIATOA

The individuals belonging to this group are distinguished from those previously described by one extremity being usually attached to some point of support, whilst the other remains free. Moreover, their protoplasm contains very frequently sulphur granules which are not crystalline. The Beggiatoa represent a type of algæ which are much more highly organised than the bacteria, and approach the group known as *Oscillatoriae*, but differ from the latter in having no phycocyanine or chlorophyll. Beggiatoæ are nearly invariably present in thermal sulphur waters, and are also constantly found in polluted and stagnant waters. They are divided into two principal classes, Beggiatoa and Crenothrix, whilst Winogradsky has added the sub-class Thiothrix. Winogradsky has made a special study of the forms of Beggiatoa in sulphur waters, and has found as many as fifteen genera and more than twenty-five different species. This author is of opinion that the sulphur granules are due to the oxidation of the sulphuretted hydrogen in the water, and that this sulphur is converted into sulphuric acid by the plant (*Annales de l'Institut Pasteur*, vol. iii., 1889, p. 49.)

BEGGIATOA ALBA (Vaucher)

Where Found.—It is very widely distributed in sewage and in drain-water from sugar factories, in thermal-sulphur springs, &c. The threads are found attached to dead insects or decayed plants, and produce white mucous flakes, which grow to considerable dimensions.

Microscopic Appearance.—The threads are from 1 to 5 μ thick, and from 3 to 4 μ long; they are bent in the shape of sinuous arcs or spirals. The free extremity is rounded, and the thread, more especially towards this extremity, contains large sulphur granules. The filament is articulated. It will grow luxuriantly at 55° C.

BEGGIATOA ROSEO-PERSICINA

Authority.—Cohn, *Beiträge*, vol. i., Heft iii., p. 157; Lankester, *Quart. Journ. of Mic. Science*, 1873, vol. xiii. p. 408.

Where Found.—In polluted ponds and ditches, covering the surface with a red or violet growth. Often noticed on the Danish sea-coasts. The filaments resemble *B. alba* in shape, but are distinguished from the latter by their reddish-violet colour. The cocci, which are produced in the filament, multiply by division, and form characteristic zoogloea masses of various shapes, branched, lobular, and netted. Rods, under certain circumstances, develop from the cocci, and, on the dissolution of the gelatinous envelope, both cocci and rods may come forth. The rods form filaments, which may be partially or entirely spiral in form. The cells composing the zoogloea are round or oval, red in colour, and are 2·5 μ in diameter.

BEGGIATOA MIRABILIS

Where Found.—In sea-water, forming white growths on decaying algæ, sea-grass, &c. Distinguished from other forms of Beggiatoa by its remarkable transverse diameter of 30 μ.

THIOTHRIX

Authority.—Winogradsky, 'Ueber Schwefelbakterien,' *Botanische Zeitung*, 1887; also 'Sur la Morphologie et la Physiologie des Sulfobactéries,' *Beiträge z. Morphologie und Physiologie d. Bacterien*, fasc. i., Leipzig, 1888; also 'Recherches physiologiques sur les Sulfobactéries,' *Annales de l'Institut Pasteur*, vol. iii., 1889, p. 49.

Where Found.—In sulphur springs along with other forms of Beggiatoa, from which Winogradsky distinguishes them by the unequal thickness of the filaments and by the production of motile gonidia.

Winogradsky, in his Memoirs, states that it is exceedingly difficult to grow Beggiatoa and Thiotrix artificially, as they die rapidly on all solid media and only develop well in waters containing sulphuretted hydrogen. He mentions incidentally that contact with distilled water rapidly kills the filaments; they become at once motionless, twist, break, sometimes swell up, and then begin to decompose. (*Annales de l'Institut Pasteur.*)

THIOTHRIX NIVEA (Beggiatoa nivea, Rabenhorst)

Authority.—Rabenhorst, *Kryptogamenflora*, vol. i., Pilze, von Winter, Leipzig, 1881; also Winogradsky, *loc. cit.*

Where Found.—Frequently in either sulphur or stagnant water.

Microscopic Appearance.—Motionless filaments surrounded by a thin sheath and attached by one extremity. This extremity, which is the base, is 2 to 2·5 μ broad, whilst the free extremity is 1·4 to 1·5 μ broad. They may reach 100 μ in length and are segmented at the free extremity, where rods 8 to 9 μ long form successively and become liberated in the liquid and exhibit motility. These are the *Gonidia*, and are never found in the true Beggiatoa. These *Gonidia* become attached, lose their motility, and then reproduce filaments.

THIOTHRIX TENUIS (Winogradsky)

Where Found.—In sulphur water.

Microscopic Appearance.—Forms very long filaments, the diameter of which is almost uniformly 1 μ only.

THIOTHRIX TENUISSIMA (Winogradsky)

Where Found.—In sulphur water.

Microscopic Appearance.—Receives the above name on account of its very attenuated filaments, which are not more than 0·5 μ broad.

CRENOTHRIX

This species differs from the Beggiatoa by the invariable presence of a sheath which surrounds the filaments; this sheath is more or less thick and has a great affinity for oxide of iron, which it fixes. No sulphur granules are found in the protoplasm.

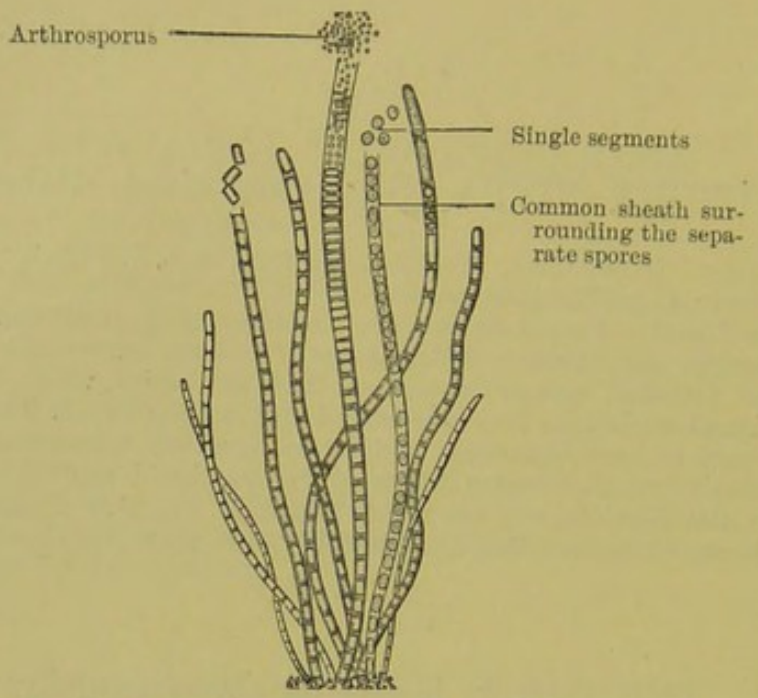


FIG. 24.—CRENOTHRIX KÜHNIANA
Magnified 600 times. (After Zopf.)

CRENOTHRIX KÜHNIANA (Crenothrix polyspora, Cohn)

Authority.—First discovered by Kühn and investigated by Cohn, and later by Zopf.

Where Found.—Found very frequently in both stagnant and running waters containing organic matter or iron. It occurs sometimes in such numbers in water-pipes that the water is unusable. It produces a thick vegetative mass in water, either brown or greenish in colour, which is due to the oxide of iron; it imparts a reddish tint to the liquid, also a disagreeable odour and a bad taste, and is capable by its presence in reservoirs or conduits of deteriorating large quantities of water at a time.

Microscopic Appearance.—It exhibits, according to Zopf, both cocci and rod forms, as well as filaments. The cocci are from 1 to 6 μ in diameter; they become invested with a gelatinous material and multiply by division, and in this manner give rise to irregularly shaped zoogloea masses, sometimes of enormous size. When cultivated in marsh-water the cocci grow into rods, which by continuous division form filaments which radiate out in all directions from the zoogloea. When this growth has attained a certain age a sheath is produced, which often contains ferric hydrate. Within the filamentous sheath the rods, by transverse division, give rise to isodiametric pieces, which become rounded and cocci-shaped. By this continuous process of division which takes place inside the sheath, such a pressure is exerted against its top end that it is forced open, and the cocci and rods escape. Sometimes the cocci and rods develop within the sheath into rods and threads, and growing through the wall of the sheath produce a number of threads, which make the original filament present the appearance of a paint-brush.

LEPTOTHRIX OCHRACEA (Kützing)

Authority.—Winogradsky, 'Ueber Eisenbakterien,' *Botanische Zeitung*, No. 17, 1888, p. 262.

Where Found.—Very frequently found in waters containing iron. Winogradsky states that the more ferrous oxide there is present in the water the larger is the number of 'iron-bacteria' found, and that the latter grow luxuriantly in waters or liquids containing very small quantities of organic matter. In vessels containing some of this water small rust-coloured flakes appear on the surface of the liquid, whilst rust-coloured patches appear on the sides of the vessel as well, the latter becoming covered, in from 8 to 10 days, with thick yellowish brown growths. Large zoogloœa masses, similar in colour, are produced on the surface and gradually sink to the bottom.

Microscopic Appearance.—The threads consist of very thin rods, which are enclosed in a more or less thick sheath, within which they occur singly or in groups. A young thread will adhere firmly to the glass with one end, the other being quite free in the liquid. At the base the sheath is many times thicker than the rodlet, whilst it gradually tapers towards the free extremity, the last 2 to 10 rodlets usually being without any sheath. As soon as the sheath becomes thick and brown, the rods either quite abandon it, or are found in the act of creeping out of it. In this manner comparatively large knotty and branched * structures are produced, consisting almost entirely of empty ochre-coloured sheaths, whilst the living threads which have generated this structure are themselves found attached to the sheath as short thin colourless little terminal branches. Multiplication takes place by detachment of actively motile rodlets, which after a time sink to the bottom and grow out into threads, formation of the brown sheath at once also commencing. Winogradsky found that the sheath only became brown in colour in waters containing ferrous oxide, and that this was effected through the oxidation of the ferrous oxide taking place in the substance of the thread itself. In the absence of ferrous oxide the threads will not grow.

* The branching is brought about by the thread breaking into two, and from one or both of the ends thus produced a new thread grows out. The place where the division took place is rendered imperceptible by the gelatinous excretion of the growing threads, so that ultimately the latter appear like thin branches on a thick brown thread, the latter being, however, generally only an empty sheath.

CLADOTHRIX (Cohn)

According to Macé (*Traité pratique de Bactériologie*, 2nd edit., 1892, p. 662, the genus *Cladothrix* is distinguished from the genus *Leptothrix* by the presence of real branchings and the entire absence of a gelatinous sheath.

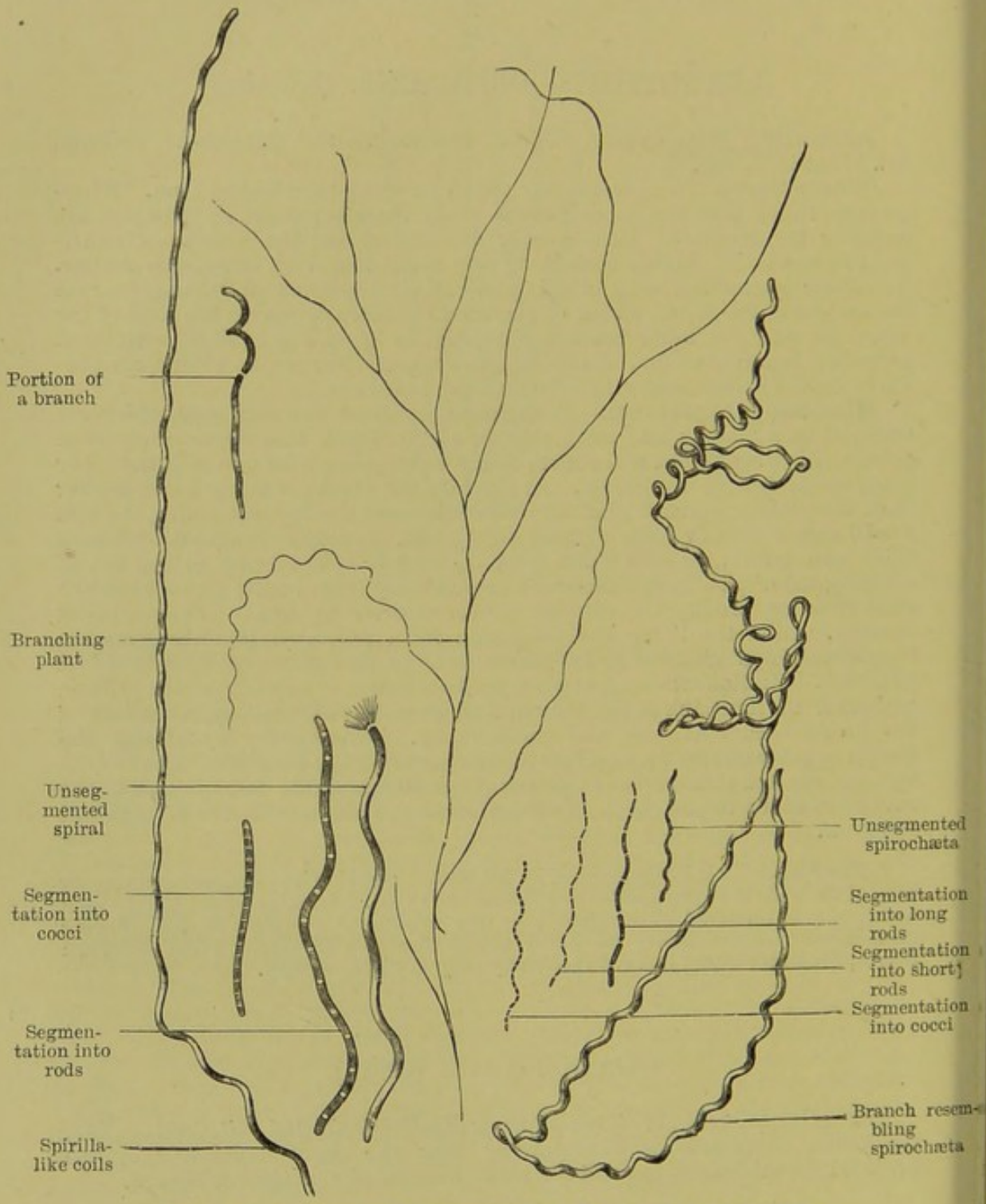


FIG. 25.—FORMS OF VEGETATION OF CLADOTHRIX DICHOTOMA.
(After Zopf.)

CLADOTHRIX DICHOTOMA (Cohn)

LIQUEFIES GELATINE

Authority.—Cohn. See also 'Sur les Caractères des Cultures du *Cladothrix dichotoma*, Cohn,' Macé, *Comptes rendus*, vol. cvi., 1888, p. 1622.

Where Found.—Present in large numbers in both fresh and brackish water, and in both running and stagnant water, especially in the latter when rich in organic material. When it is present in large numbers in water it gives rise to whitish flocculent masses.

Microscopic Appearance.—Long motionless filaments, about 0·4 μ broad and reaching sometimes a millimetre even in length. Distinct branching of the filament is visible. The contents are hyaline. It is straight or sinuous, sometimes undulatory or spiral. The filaments separate up into isolated segments either straight, or comma-, or spiral-shaped, endowed with motility, and with a tendency to unite together to form small flaky zoogloea masses.

Cultures.—

Gelatine Plates.—Forms in four to five days very small yellowish dots, which are surrounded by a brown halo which extends more and more over the gelatine. On reaching the surface it appears as a small brownish button, sometimes having a white efflorescence surrounded by a very brown halo, and a depression due to the slow liquefaction of the gelatine, which it causes. (Macé.)

Gelatine Tubes.—Forms a thin greyish expansion which slowly liquefies the gelatine. The liquid remains clear, but acquires a brown colour, and a quantity of flocculent material collects at the bottom. (Macé.)

Agar-agar.—Forms at 35° C. a shining thick expansion, which adheres so closely to the agar that it is impossible to remove it without also carrying away portions of the agar. The expansion exhibits a great tendency to form concentric rings. Sometimes the growth becomes covered with a greyish efflorescence, which is dry and very brittle. The agar becomes strongly brown in colour. (Macé.)

Broth.—Forms light whitish flakes with a radiated appearance. The liquid remains clear, but becomes brown in colour. It grows rapidly at 37° C. (Macé.)

Remarks.—All the cultures have a very strong, mouldy smell.

CLADOTHRIX INVULNERABILIS

LIQUEFIES GELATINE

Authority.—Acosta and Grande Rossi, 'Descripción de un nuevo Cladothrix, Cladothrix invulnerabilis,' *Centralblatt f. Bakteriologie*, vol. xiv., 1893, p. 14.

Where Found.—In water.

Microscopic Appearance.—The microscopic appearance is not given in the *Centralblatt.*

Cultures.—

Agar-agar.—In forty-eight hours small circular colonies of a dirty-white colour are visible both along the needle's path in the depth and on the surface. They cling so tenaciously to the agar that during their transference portions of the latter are removed with them. Later the growth becomes silver-white, and at a more advanced stage yellowish, and in about fourteen days appears as a star with five to six rays, the centre exhibiting a small circular depression surrounded by five to six protuberances and depressions.

Glycerine-agar.—Grows rapidly and abundantly.

Gelatine Tubes.—Produces a silver-white colony, depressed in the centre but raised at the periphery. It resembles the growth in agar, but the gelatine begins to liquefy slowly in the course of a few days, and growths attach themselves to the sides of the tube and to the edge of the fluid gelatine.

Potatoes.—Grows rapidly and abundantly, forming in forty-eight hours a broad band where the needle has streaked the surface, consisting of small white confluent chalk-like colonies, emitting an odour of damp soil. The potato becomes blackish in colour.

Milk.—Forms a solid yellow layer on the surface, so that on inclining the tube nothing runs out. Beneath this layer is a transparent liquid, underneath which is seen the milk.

Cocoanut Milk.—Abundant cloudy growth.

Broth.—Abundant cloudy growth.

Sterilised Water.—Abundant cloudy growth.

Remarks.—When heated to 120° C. its development is retarded for six days, and the growth is impaired. It can withstand six successive exposures to intermittent sterilisation at 100° C. Potato cultures exposed for ten minutes to an electric current of fifty cells were not destroyed. It will grow equally well in the absence as in the presence of air.

CLADOTHRIX INTRICATA

LIQUEFIES GELATINE

Authority.—Russell, 'Untersuchungen über im Golf von Neapel lebende Bacterien,' *Zeitschrift f. Hygiene*, vol. xi., 1891, p. 191.

Where Found.—In sea-water mud, and by Russell included under the genus *Cladothrix*.

Microscopic Appearance.—Varies according to the nature of the medium in which it is cultivated, its normal appearance being apparently seen when grown on gelatine. In this medium it exhibits long, slender cells with homogeneous contents united together to form long filaments. When stained with Kühne's carbolic methylene blue (see p. 46) the divisions of the filament are shown very clearly. In potato cultures the cells are shorter and have rounded ends, and as the age of the culture increases the cells divide up into several short plump individuals, in many of which a slender, oblong spore is visible. It is usually motionless, but now and again distinct motility may be observed.

Cultures.—

Gelatine Plates.—In from twenty-four to thirty-six hours small white lustrous mould-like colonies are visible to the naked eye. Under a low power the contents consist of a thick network of threads, from which a number of twisted and interwoven filaments extend into the surrounding gelatine. These filaments grow rapidly, and liquefaction of the gelatine soon ensues. If a stained microscopic preparation be made of one of the filaments, 'false' branchings are visible, due to the individual cells at various points of the filament becoming partially detached and displaced. These cells in pushing up against one another get bent out of the straight and grow out into the gelatine, although still attached to the original filament by intercalary growth which extends throughout its whole length. In this way Russell accounts for the appearance of what he considers to be the pseudo-branching of the filament. The pseudo-branches are often spirally intertwined.

Gelatine Tubes.—Grows very rapidly, and in twenty-four hours finely-twisted horizontal threads extend all along the needle's path in the depth. The gelatine is quickly liquefied.

Agar-agar.—Forms an abundant but thin, dull, white expansion, from which fine threads extend into the depth.

Potatoes.—Forms an irregular, dull, white expansion, which does not increase in size after three days.

Broth and Sea-water Broth.—Forms an abundant deposit, which on shaking breaks up into small pieces.

STREPTOTHRIX FOERSTERI (Cohn)

LIQUEFIES GELATINE

Authority.—Gombert, *Recherches expérim. sur les Microbes des Conjonctives à l'Etat normal*, Montpellier, 1889. Also Almquist, 'Untersuchungen ueber einige Bacteriengattungen mit Mycelien,' *Zeitschrift f. Hygiene*, vol. viii., 1890, p. 195. Also Gasperini, 'Recherches morph. et biol. sur un Microorgan. de l'Atmosphère, le Streptothrix Fœrsteri,' *Ann. de Micrographie de Miquel*, July and August, 1890. Also G. Roux, *Précis d'Analyse microbiologique des Eaux*, Paris, 1892, p. 369.

Where Found.—Found first in the concretions of the lachrymal duct by Fœrster, Cohn, &c.; it has also been found in air, and in salt and fresh water by Almquist and Roux. Roux assumes the Streptothrix found in water by him and Almquist to be identical with that described by Gombert. Almquist states that his Streptothrix did not liquefy gelatine.

Microscopic Appearance.—Long filaments with rounded ends, rectilinear, or more often undulatory or spiral-shaped. From 0·5 μ to 0·6 μ broad and extremely variable in length, from 4 μ and 6 μ to 92 μ, and sometimes longer. Forms spores 0·8 μ in diameter. Not properly motile. (Gombert.)

Cultures.—

Gelatine Plates.—Forms in four or five days small greyish white cloudy spots in the depth of the gelatine. They have an opaque dark grey centre, whilst the periphery is white and semi-transparent. The gelatine is depressed in the vicinity of the colony. On endeavouring to detach a portion of the colony with a platinum needle the whole is removed. Under a low power they are blackish in colour, and the periphery consists of a number of short, irregular, and stiff hairs, recalling the appearance of a chestnut in its spinose capsule. The surrounding gelatine does not become brown, as is characteristic of the growth of *Cladothrix* cultures. Slow liquefaction of the gelatine ensues. (Gombert.)

Agar-agar.—Grows abundantly and rapidly at both 20° and 37° C. A whitish thin crust forms over the growth, which loses at once its white colour when washed over with liquids. (Almquist.)

Broth.—Forms at the end of a few days at 37° C. white spinose spherules, which float in the liquid or attach themselves to the walls of the tube. The liquid remains clear and becomes slightly darker, but never assumes the brown tint characteristic of *Cladothrix dichotoma*. (Gombert.)

Sterilised Tap-water.—Grows fairly well.

Remarks.—Not pathogenic to animals (Gombert, Gasperini, and Roux).

FUSARIUM AQUAEDUCTUUM (Fusisporium moschatum)

LIQUEFIES GELATINE

Authority.—Rabenhorst and Radlkofer described it under the name of *Selenosporium aquaeductuum*. Eyfert, 'Zur Entwickelungsgeschichte des Selenosporium aquæductuum Rabenhorst und Radlkofer,' *Botanische Zeitung*, 1882, p. 691. Kitasato, 'Ueber den Moschuspilz,' *Centralblatt für Bakteriologie*, vol. v., 1889, p. 365. Heller, 'Zur Kenntniss des Moschuspilzes,' *Centralblatt für Bakteriologie*, vol. vi., 1889, p. 97. Von Lagerheim, 'Zur Kenntniss des Moschuspilzes,' *Centralblatt für Bakteriologie*, vol. ix., 1891, p. 655.

Where Found.—Found by Eyfert in enormous numbers on mill wheels and turbines in Brunswick, actually impeding the motion of the wheels. He also mentions that the intensely strong aromatic smell which it generated was so penetrating as to give the men working in the mill headaches. Found by Kitasato in hay infusions; found by Lagerheim in the tap-water of Upsala, forming large greyish white slimy masses, which hung down like long rags from the opening of the pipe. It is also sometimes pale pink and brownish in colour.

Microscopic Appearance.—The spores are semilunar, or meniscus-, also sausage- or club-shaped, and are about 7 μ to 13 μ long and 1 μ to 1·5 μ broad, each spore possessing three to four transverse walls, which, however, are not always equally distinct. These spores, when dried and preserved for five months, will still grow. Highly refractive bodies are visible in the spore, which must be regarded as small drops of oil. The spores are easily stained with aqueous solutions of aniline colours, but the latter are removed by the usual decolorising agents. They are also stained by Gram's method. At 15° to 18° C. in from twelve to fifteen hours a tube-like extension is visible, proceeding from the pointed end of the sickle-shaped spore, which in eighteen hours is three to four times as long as the spore, and exhibits numerous small swellings indicating the commencement of the branching of the filament. These filaments grow rapidly, producing lateral branches, and later small mould centres, which quickly increase in size. The spores are produced from the filament both laterally and terminally. (Lagerheim.)

Cultures.—

Gelatine Tubes.—In three to four days forms a cloudy growth consisting of masses of fine interwoven threads, at the edge of which the individual threads are distinctly seen. The central portion becomes more and more opaque, and assumes first a white, then a yellow, and finally a red colour. In consequence of slight liquefaction the colony sinks somewhat into the gelatine.

Agar-agar.—Grows as in gelatine, but without liquefaction.

Potatoes.—Forms a white, star-shaped expansion, which becomes larger and thicker, and gives rise to delicate, bundle-shaped ramifications, which extend over the surface. As the culture becomes older the white colour gives place to pale pink, and finally vermilion.

Broth.—Produces masses of flocculent material of a dirty grey colour, which collect at the bottom of the tube.

Remarks.—It grows best at 15° C., very slowly at 3° C., and not at all at −5° C. The spores are destroyed at 38° C. in forty-eight hours. The spores will germinate very readily in distilled water. It is strictly aërobic. It is pathogenic to frogs when subcutaneously injected (Heller). In all cultures a strong odour of musk is produced.

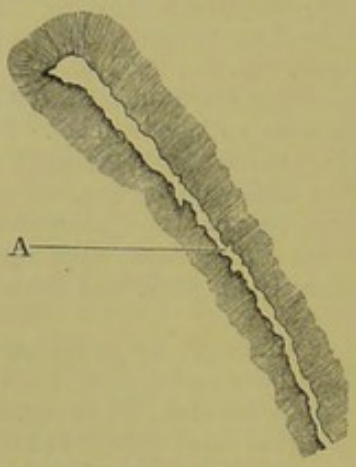


FIG. 26.—FUSISPORIUM MOSCHATUM.

Appearance in gelatine-cultures 8 days old. Natural size. A. Red central portion. (After Heller.)

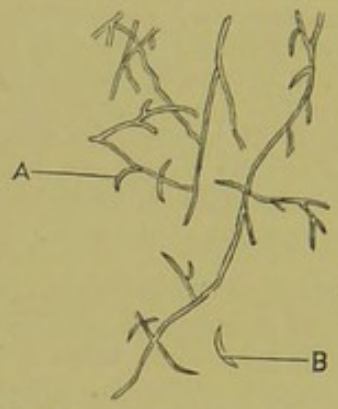


FIG. 27.—FUSISPORIUM MOSCHATUM.

Growth on gelatine plates. Magnified 100 times. A. Spore in the act of breaking off. B. Spore. (After Heller.)

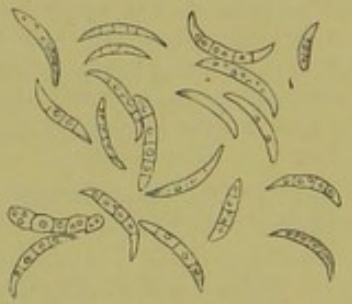
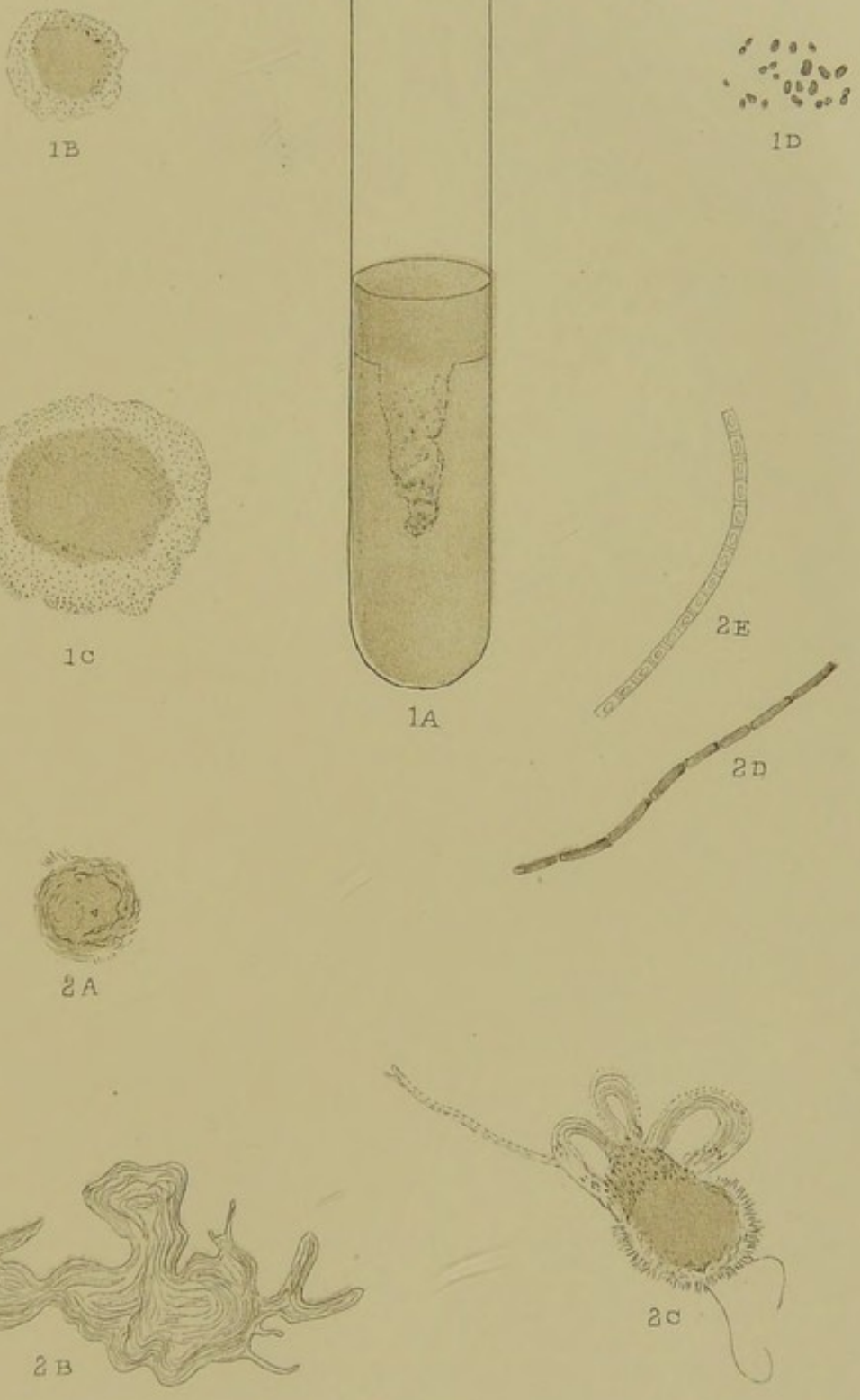


FIG. 28.—FUSISPORIUM MOSCHATUM.

Spores taken from a gelatine-culture. Magnified 300 times. (After Heller.)

Plate . I .



1.Bacillus prodigiosus .
2.Bacillus subtilis .

G.C.Frankland del.

Hanhart imp.

Plate II.



2B



1A



2C



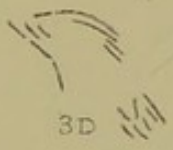
3A



3A



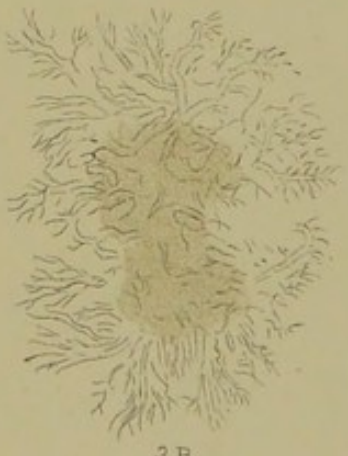
3E



3D



3C



3B

1. Bacillus ramosus. G.C. Frankland del. Hanhart imp.
2. Bacillus violaceus. "
3. Bacillus arborescens.

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The names written in *italics* indicate that a tabular description of the organism is given in the Appendix.

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