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VEGETABLE DECOMPOSITION IN DITCH WATER SIMULATING SEWAGE CONTAMINATION.

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(With Plate V.)

LATE in the autumn of 1922, following an exceptionally dry period, the water of some of the ditches in the "Backs" at Cambridge became foul, so that instead of looking dark and clear, as it usually does, it became milky in appearance, and gave off an offensive odour so like that of sewage that many people believed that it had become contaminated by leakage from the sewer near by. This led to an investigation, which proved that no pollution with sewage was taking place, and that the trouble was due solely to vegetable decomposition, aided by stagnation of the water, the result of an unusually dry season.

Since the possibility that decay of leaves in stagnant water may simulate contamination with sewage does not seem to be widely recognised, and since such an occurrence is liable to alarm people unnecessarily, and, perhaps, to lead to useless expense, it seemed desirable to record this experience. Consequently in the following paper there is given a short account of the state of the ditches during the period when they attracted attention on account of their objectionable state, and of the bacteriological observations which were made with the water.

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Attention began to be directed to the ditches about the end of October, 1922, when the odour emitted by the water became the subject of much complaint. It resembled, indeed, very closely that of sewage. Hydrogen disulphide was clearly distinguishable. This gas may, of course, be detected whenever the mud at the bottom of any ditch or pond is disturbed, but here it was evident without any disturbance of the mud, and to it were added other odours which give to the smell of sewage its distinctive characters.

The appearance, also, of the water lent support to the theory of sewage contamination. Bubbles occasionally appeared on its surface; but these were, on the whole, not much in evidence. A much more striking change was pronounced milkiness, which would appear from time to time like a cloud in the water, now in one place, now in another, and, after lasting for a week or more in any one region, would slowly fade away to appear elsewhere. This appearance was very striking, and it is scarcely an exaggeration to say that, at its height, the previously clear black water looked as if a can of milk had been emptied into it. It came and went, as has already been said, and the sewage-like odour varied with the milkiness.

The low-lying land on the west side of the river Cam where it flows past some of the colleges from Queens' to St John's, is divided up into a number of paddocks, each of which is surrounded, in the seventeenth century Dutch manner, by ditches, similar to those seen in the well-known picture by Hobbema, in the National Gallery. The ditches open, for the most part, directly into the river. There is seldom any perceptible flow of water in them, but at ordinary times they are clean, and filled with water which is clear, or, at worst, covered with duck weed. They are overhung with trees—limes, elms and chestnuts; and in the autumn they get filled with leaves from these trees and the many others around them.

On the far side of the paddocks, running parallel to the river, is the Queen's road, under which is a deep sewer. On the further side of the road the ground, which here rises a little, was, before the war, occupied by college gardens and cricket fields. But on one of the latter, there grew up the First Eastern, Military Hospital, and, its buildings are now temporarily occupied by a civil population of 200 families. The change in the water was first observed in the ditches which lie at right angles to the river, on either side of Garret Hostel Lane, and especially at their ends furthest from the river, and nearest the Queen's Road, where the drain from these dwellings joins the main sewer under that road. It was therefore not unnatural to suspect that a leak had occurred in the sewer at this point, especially as no one remembered to have seen the ditches in such a state before, and the exceptionally dry summers[1] were known to have caused shrinkage of the gault and subsidence of the foundations of some of the houses in the neighbourhood; and it was thought that the shrinkage might have affected the sewer also[2].

Thus it was not without cause that sewage contamination was suspected, in spite of official denial. I confess that I myself held this view, until convinced by personal investigation of its untenability, although the Borough Surveyor assured me that the sewer was intact, and that even if there were a leak the sewage which escaped could not possibly get into the ditches owing to the greater depth of the sewer.

And there were other factors, undervalued by me at the time, which did not fit in with the theory of sewage pollution. For already in the autumn of

[1] The summer of 1921 was so exceptionally hot and dry that it will not easily be forgotten; wells and streams around Cambridge dried up, and the lack of water in country districts became severe. The winter which followed did not make up the deficiency, and was followed by a hot and dry spring and early summer. There was, it is true, a good deal of rain in July 1922, but less in amount in Cambridgeshire than in most other parts of the country. It was not sufficient to fill up our wells, and after the very dry autumn which followed, the shortage of water in Cambridgeshire was worse than ever. A countryman told me that his well, which had held out through 1921, had become dry in the autumn of 1922.

[2] The sewer, however, at this point does not lie in the gault, but in a bed of sand and gravel of considerable thickness overlying the gault.

1921, the ditches in question had attracted unfavourable notice for the same reasons as drew so much attention to them a year later. But the trouble did not then attain to such proportions as it did afterwards, and no great complaint was made. The Colleges concerned were, however, requested to clean out their ditches, which they accordingly did, and the water remained clean during the following summer.

Thus the trouble was recurrent; it occurred each time, after the fall of the leaves in the early winter, and not in the summer when sewage contamination, if it took place, might be expected to cause most offence. It ceased after the ditches had been cleaned out.

Investigation was first made of the extent and distribution of the trouble, and directed to finding its points of greatest intensity, if any. The local condition of the water was plotted on a six inch map, and the variations which occurred from time to time were recorded.

The ditches out in the open country around Cambridge were examined and found nowhere to be in a state similar to those in the "Backs," even when they were full of leaves[1]. In the "Backs" themselves the ditches were not all equally affected; and it was the fact that the maximum pollution seemed to coincide with the points most likely to be reached by sewage escaping from the drain in the Queens' road that lent such probability to the view that the nuisance was traceable to the sewer.

But, on the other hand, there was a ditch just outside the town, namely on Coe Fen and bordering the Leys School grounds, which was even more affected than the Garret Hostel ditches; and there did not seem any possibility that this ditch could have been polluted with sewage.

Such was the ambiguous state of the investigation when, in the middle of December, the Commissioners of the Cam, at the request of the Mayor, kindly consented to lower the level of the river, so that the ditches might be emptied of their water. While they remained in this condition, for a period of nearly a week, they were carefully inspected by the Medical Officer of Health, Dr Laird, and myself to see if any signs of sewage running into them could be detected. We had already determined, as I have said, on the most likely spots, and these were subjected to a thorough examination. But no trace of sewage could be detected.

When the river was allowed to rise again to its normal level the ditches became filled with clean water once more; and copious rain falling about this time, some movement was re-established in them. They have since then remained in a satisfactory condition[2].

Thus the result of this investigation was to convince us that no leakage of sewage into the ditches was taking place, and that the cause of their foul condition must be looked for in other directions. It remained to be seen whether

[1] Since this was written, a ditch far away in the country and which could not possibly contain sewage from any human habitation was found in a similar condition. This was in July 1923.

[2] A very slight return of the conditions described was noticed by me in the summer of 1923.

the bacteriological investigation which was then in process would reveal the cause of the unusual condition of the water.

Bacteriological Investigation

Samples of the water were taken in sterile Winchester quart bottles from the ditches on various occasions and removed to the laboratory.

An attempt was first made to determine whether the milkiness of the water was due to a cloud of bacteria, or to chemical matter in a fine state of division suspended in it. Samples put up and examined in the hanging drop under a 1/12th oil-immersion lens showed numerous bacteria, but it was difficult to decide whether these were present in numbers sufficient to account for the appearance. Sterile tap water, contained in one-litre flasks, and to which a tube or two (about 10 c.c.) of nutrient broth had been added, became, when sown with some of the milky water and incubated for a day or two, as cloudy as the ditch water itself. And so also did similar flasks sown with certain cultures which were isolated from the ditch water, and which I shall presently describe. After due consideration I am inclined to the view that the turbidity of the ditch water was directly due to the cloud of bacteria in it, and not to finely divided non-living matter in suspension. The cloudiness did not disappear on the addition of acid.

Plate cultures on ordinary nutrient agar yielded various bacteria. It was significant that *B. coli* was not recognised among them. Two mono-flagellate, round ended, short bacilli, however, at once attracted attention on account of the characteristic odour produced by their cultures. These became known in the laboratory as S. 1 and S. 2, and under these names will be described here.

Two Bacilli isolated from the Water of the Ditches, and capable of causing Foul and Characteristic Odours.

These two bacilli have many properties in common: They produce a copious, confluent growth on various kinds of media, including McConkey's bile-salt agar; they are Gram-negative, stain rather feebly with various dyes, and form acid but no appreciable amount of gas; they are actively motile, and each kind is provided with long, single, terminal flagella. They grow at various temperatures from 3° C. to nearly 40° C. Perhaps their most notable feature is their variability of form which, under favourable conditions of temperature may be an oval bacillus, and at higher temperatures long curved rods and undulating threads.

While the most important characters of these bacilli have thus been briefly recorded it will no doubt be desirable to give in addition a more detailed description of them, and to refer particularly to the points wherein S. 1 differs from S. 2.

Cultural Characters.

Growth of either species is plentiful and fairly rapid at all temperatures from 9° C. to about 34 or 35° C. At 3° C. it is slow, does not appear for some days, and never becomes luxuriant. As the temperature approaches the opposite limit growth again becomes impaired; at 37° C. it is distinctly scanty, and it ceases altogether before 40° C. is reached.

On nutrient agar, from 9° C. to 34° C. each species forms a copious, moist, pearly grey, translucent growth, very like that formed by *Vibrio cholerae* or one of the bacilli included in the typhoid-coli group. The growths of S. 1 and S. 2 are not precisely alike; often there is very little difference between them, but on the whole that of S. 1 tends to become a shade thicker and whiter than that of S. 2.

This difference, slight though it is, comes out better in agar plate cultures, when separate colonies are given plenty of room to develop. Under these conditions S. 1 grows in rather large thick whitish colonies which, when there are not more than 20 or 30 to the Petri dish, may be as much as 5 or 6 mm. in diameter. S. 2 under the same circumstances produces slightly smaller and thinner colonies. Thus if, in respect to their colonies on agar, S. 1 may be compared with *B. coli*, S. 2 may be said to resemble rather *B. typhosus*. Both are moist, and free from stickiness as a rule, but when grown at 37° C., and therefore producing long threads (which one imagines may possibly become entangled together) they are somewhat mucoid; and old cultures of S. 2, even when grown in the cold, show a tendency to draw out into a thread when the platinum needle which has touched them is being withdrawn. It is tempting to connect this with their habit of forming capsules, indications of which have been observed and will be described in a moment.

On serum growth is less copious than on agar.

On potato it is luxuriant, a thick pale cream coloured film being produced in 24 hours.

In broth growth appears as a cloud which is appreciably denser in the upper layers, and, in the early stage, a ring of denser coherent growth may occur where the surface of the fluid is in contact with the walls of the tube—a ring similar to one which I have often seen in cultures of *V. cholerae*.

In gelatin-shake cultures of either bacillus growth is confined for the most part to the surface and upper layers of the medium. Colonies in the substance and visible to the naked eye are practically confined to the upper half centimetre and rapidly fall off in size the deeper they penetrate. At first sight the rest of the gelatin seems to be free from growth, but close inspection in a good light, and particularly with the aid of a hand lens, reveals minute colonies extending to the bottom of the tube. This is a constant phenomenon and repeats itself when the cultures are sown from single colonies. It is rather more obvious with S. 1 than with S. 2.

No gas bubbles appear. There is, of course, the usual disagreeable odour, and therefore presumably gas, but, obviously, it is not produced fast enough to become entangled in the gelatin and to collect into bubbles.

The difference in the surface colonies of S. 1 and S. 2 on gelatin, again as on agar, are best seen in plate cultures. Growth is slow and the colonies continue to increase in size for several days. Those of S. 1 become, when moist, strongly raised and of a pearly white colour, reminding one of the hemispherical colonies of the bacillus of Friedländer. At their best they attain a diameter of 4 or 5 cm. When old and dried they may have a raised centre and a thinner indented margin. Those of S. 2 appear as white spots of smaller size lying in the centre and at the bottom of cup-shaped depressions in the gelatin. These depressions are sharply defined and circular. They contain little or no fluid, the liquefied gelatin being, apparently, absorbed by the surrounding jelly.

The deep colonies of S. 1 are not distinguishable from those of S. 2, or at most are only very slightly larger and whiter; both are lenticular in shape and small in comparison with the colonies on the surface.

In gelatin stab cultures S. 1 forms a large raised white colony at the surface, S. 2 a smaller colony, thinner and greyer. Down the track of the needle, there is growth only for a few millimetres after which the visible track fades rapidly away.

There is a very slight greenish florescence seen sometimes in the upper part of cultures of S. 1 but not in those of S. 2.

The liquefaction of S. 2 begins at the surface and proceeds but slowly. After 24 hours a little cup-shaped hollow is just visible, and next day the appearance is rather like that of a typical cholera culture. After a week or 14 days liquefaction has only extended about a centimetre or two from the surface.

In 1 per cent. peptone water both species grew well, and if glucose be added they produce acid but no gas. The fluid in the Durham's tubes, inserted to catch gas should any be formed, retains its blue colour for days, showing that the glucose there is not fermented, and that the microbes will not grow under the anaerobic conditions which prevail there. When lactose is the only sugar present no acid is formed, but the medium, to which litmus has been added, may become bleached in its deeper part.

It seems probable that these bacilli prefer weak solutions of organic matter to strong ones as culture media. In water to which only one or two per cent. of nutrient broth, or the same quantity of the boiled meat extract used for making the broth, had been added they grew well, and produced a marked opalescence or turbidity comparable to that of the ditch water seen under similar conditions of bulk and light. In gelatin (10 per cent.) great reduction of the amount of nutrient broth added did not seem to make any difference to the amount of growth.

On McConkey's bile-salt agar these microbes grow freely—in which point again they resemble *V. cholerae.*

Microscopic Characters.

One of the most notable characters possessed by these bacilli is their variability in size and shape on different media and especially at different temperature. It will therefore be necessary to describe their microscopic characters rather closely.

But not only do these bacilli vary under the conditions named above but their length and appearance is not quite constant on any given medium or at any given temperature. This seemed to be due to slight and uncontrolled variations in different batches of the same kind of medium. Thus one set of potatoes would grow longer bacilli than others. The same was true of different batches of serum or even of agar, and it seemed to be generally the case that when the growth was scanty the bacilli were longer, and when it was copious they were shorter, as though qualities in the medium favourable to massive growth promoted also rapid cell division, so that long individual bacilli tended to disappear. We shall see that when the temperature of cultivation is raised to 37°, at which level growth is always scanty, much longer forms are the rule. On account of this variability the measurements given below must be taken as approximate only, and the descriptions as applicable to average specimens.

On ordinary nutrient agar at 10° C. or 23° C. both species appear, for the most part, as oval bodies, separated from one another by rather wide intervals. They measure 1·5 to 2μ in length and are about half as broad as they are long. A few longer forms may appear. At unfavourably low temperatures, *c.* 3° C., S. 1 grows in distinctly longer forms. They both stain feebly with methylene blue, or even with methyl violet, unless the staining is prolonged, and they usually appear as a dark outline with pale interior. A few longer forms, as already mentioned, may be seen. These are apt to stain more darkly.

Occasionally there may even be a thread (commoner in S. 2 than in S. 1) such as one sees in cultures of *B. typhosus,* and foreshadowing what may occur at higher temperatures.

With carbol-fuchsin they show polar staining, and in longer forms, such as occur in cultures about to be described, the stainable material is broken up by the violence of the stain into a number of segments[1]. Such appearances have probably no relation to the normal structure of the bacillus.

On some potatoes they grow, at 22° C., as definite bacilli three or four times as long as they are broad, and 3 or 4μ in length, round ended and somewhat curved, and only occasionally arranged end to end. On other potatoes especially when growth is very luxuriant they are shorter and approximate to those seen on agar cultures grown at similar temperatures.

The bacilli grown on broth or serum are similar to those grown on potato, and are, on serum especially, equally variable.

When the temperature of incubation is raised to 30° C. the bacilli, even on agar, grow longer and approximate to those grown on potato. On raising the temperature, very little change takes place until the region of 37° C. is reached. At that temperature, when growth is as we have said, scanty, the bacilli, after 24 hours' incubation, are long curved rods 8 or 10μ in length, and if the temperature of incubation be approximated to 40° C. involution forms appear, long undulating threads, and sometimes strings of ill-shaped sausage-like bacilli, or threads swollen and staining more feebly than the others and with little sparsely scattered darkly stained granules adhering to their cell membranes.

These descriptions all refer to 24 hour cultures at the temperatures mentioned. When grown longer no important changes were observed. No polar bodies or spores were seen at any time. The bacilli, in spite of the absence of spores, remain alive when left in the cupboard for many months.

In some of the microscopic preparation, especially when stained darkly with methyl violet, there was a definite suggestion of capsules (see Fig. 2).

[1] A similar appearance in *B. coli* and *B. typhosus* may be seen in Figs. 56 and 61 in Mallory and Wright's *Pathological Technique.* It is not stated what stain was used.

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The flagella, stained by Stephen's modification of Van Ermengem's method, were single and approximately terminal. They showed, for the most part, three or two and a half complete undulations, and appeared considerably longer than those of *V. cholerae* and more like those of *B. pyocyaneus* except that the undulations were more pronounced.

Taking all their characters into consideration these bacilli seem to be akin to *V. cholerae*; and from their Gram-negativeness, their incapacity to form an appreciable amount of gas from various sugars, their habit of forming under certain circumstances long undulating threads, and more particularly, from their possession of single terminal flagella they may, I think, be regarded as belonging to the family of spirilla.

S. 1 produces in artificial cultures an unpleasant odour, not unlike that of *B. coli*. S. 2 produces a more offensive smell in which a suggestion of putrefaction is combined with the smell of over-ripe quinces. Both are capable of producing a cloud in water to which a very small amount of organic matter (*e.g.* 1 per cent. of boiled meat infusion) has been added.

For these reasons I believe that the bacilli in question, but especially S. 2, were mainly responsible for the abnormal milkiness of, and the offensive odour emitted by, the water in the ditches.

Probably other micro-organisms contributed as for example by producing H_2S. No such micro-organisms were isolated from the ditches; but much remains to be done with investigation of the anaerobic bacteria present in the water.

Chromogenic Micro-organisms.

Some chromogenic micro-organisms found in the water, and probably the cause of certain appearances of colour in the contents of the ditches which I have not previously alluded to, deserve a few words of description.

Shortly before the water was drawn off from the ditches my attention was called to certain patches of pinkish or violet colour which were making their appearance here and there on the leaves and twigs lying at the bottom of the water, and on the boards which line the sides of the ditch on the East of St John's Wilderness. This colour became still more evident on the wood when the water was drained away from the ditch. It was obviously caused by a thin layer of some alga-like growth. Samples were collected of the more brightly coloured of the leaves and sticks, brought to the laboratory, and put, together with ditch water, in large covered glass dishes. In the course of a day or two a violet coloured micro-organism was found to be growing in indefinite patches, an inch or more wide, at the bottom of one of the glass dishes. Numerous efforts were made to get this to grow in pure culture, but all failed, although, among others, a medium made of a decoction of leaves and ditch water was employed.

The micro-organism was at first thought to be *B. violaceus*, but though I had no difficulty in cultivating this latter micro-organism from the ditch water, that which we saw growing in the coloured patches was of another order.

Under high magnification it was found to consist of little curved, round-ended, sausage-shaped bodies, on some of which could be seen a single wavy flagellum. They were about as long as the diameter of a red blood corpuscle, and probably almost as deeply coloured, for their colour was quite apparent even when seen under the oil-immersion lens. They appeared to have no nuclei or spores, unless indeed little colourless highly refractile bodies of which several, or in some, many, could be seen in each cell, were of this nature. Sometimes the coloured contents of the cell did not extend quite to its rounded extremities, as if it had shrunk away a little from the envelope. Occasionally two cells were seen end to end. These flagellates were found, not only when growing on the glass bottoms of our dishes, but on the coloured leaves and twigs, and there can be little doubt that they were the chief, if not the sole, cause of the strange appearances of colour noticed in the ditches.

I do not think that *B. violaceus* played any part in these appearances, though, as already said, it was readily isolated from the water in pure culture.

Apart from the coloured patches of growth just referred to, the water itself, sometimes and in certain places, seemed to be tinged with a similar violet-pink colour, and on one occasion, in the laboratory, a litre flask of unsterilised ditch water to which had been added some meat infusion, and which had been sown with S. 1 and S. 2, turned distinctly violet; but I was unable to isolate any coloured bacteria from it; nor did a flask of similar water turn violet after being sown with *B. violaceus*.

Summary and Conclusions.

In autumn and early winter certain ditches around Cambridge became offensive, and their water turned milky. This led to a grave suspicion of sewage contamination, which, however, was satisfactorily disproved. On the other hand certain bacilli with single flagella were isolated from the water of the ditches and found capable of giving off from their artificial cultures an odour comparable to that of the ditches. And to these bacilli, acting on the dead leaves which found their way into the water, and, probably, in conjunction with other anaerobic bacteria which were generating hydrogen disulphide, the sewage-like odour is attributed.

Certain patchy changes of colour in the leaves and wood immersed in the water are attributed to a chromogenic flagellate which was found growing on them.

DESCRIPTION OF PLATE V.

Fig. 1. S. 2, from an agar culture, stained by Stephen's modification of van Ermengem's silver nitrate method. S. 1 is similar.

Fig. 2. S. 1, from a 24 hour growth on agar at 22° C. stained darkly with methyl-violet, showing indications of capsules.

Fig. 3. S. 1, from a 24 hour growth on agar at 36° C. stained darkly with methyl-violet. The granules are probably artifacts caused by overstaining; they were not seen in lightly stained specimens.

Note. My thanks are due to Dr M. R. Swann, for kindly taking the photographs that are reproduced in this plate.

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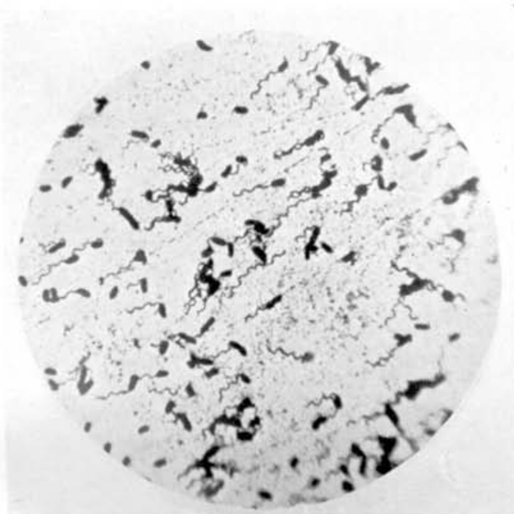

Fig. 1

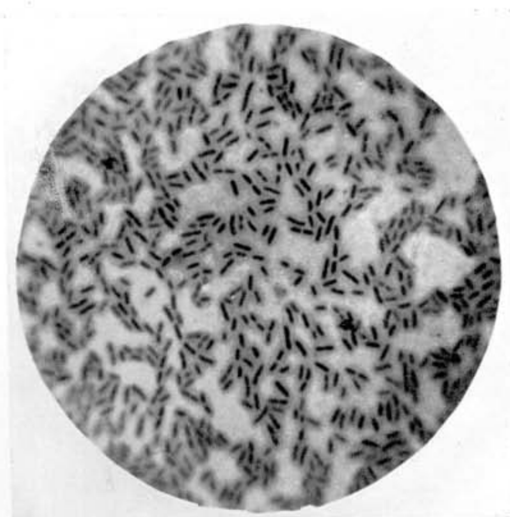

Fig. 2

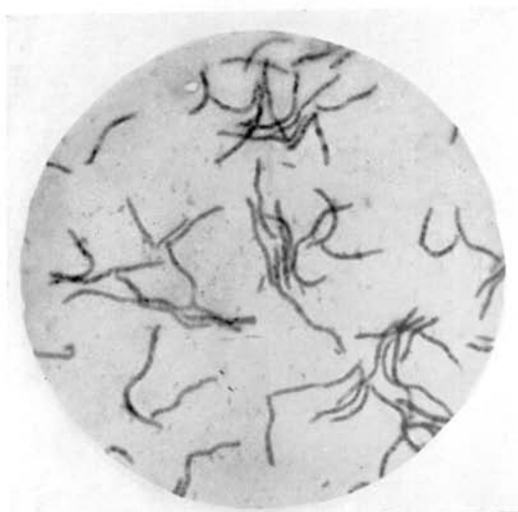

Fig. 3

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