MICROSCOPIC EXAMINATION OF A GELATINOUS GROWTH

FOUND IN A SMALL POOL IN CONNEXION WITH A SPRING IN THE GROUNDS OF RED LODGE, BASSETT.

By T. W. Shore, M.D., B.Sc.

The gelatinous material, when received by me, was contained in water, and on opening the bottle I perceived a distinct, but not highly marked, odour suggestive of slow decomposition. To the naked eye, the material was a semi-transparent, soft, gelatinous substance, mostly colourless, but here and there of a green appearance. It chiefly took the form of hollow globular masses of various sizes, broken away at one part, or of hollow cylinders. The inner surfaces of these masses were irregular, shreddy and softer than the outer surfaces, which were smooth, more consistent, and more opaque than the rest of the mass. The thickness of the substance varied from an eighth of an inch to half an inch. One or two pieces consisted of long rods of gelatinous substance, growing perfectly symmetrically around a blade of grass which acted as a sort of central core. At first sight, these appearances suggested a gelatinous Alga.

On making a preliminary rough microscopic examination by teasing and pressing out a piece in water on a slide, no particular structure was seen with a low power, but with a one-sixth inch objective, it at once became apparent that there was a general structureless ground substance, quite homogeneous, and having imbedded in it immense swarms of micro-organisms of every size, shape, and form, and belonging to a large number of species of those fungi which are

known as *Schizomycetes* or " Bacteria." Here and there, were found a few chlorophyll-containing unicellular Algæ, mostly species of *Protococcus* and *Pleurococcus*. There were also seen, here and there, amœboid masses of granular protoplasm, possessing nuclei, but whether these were ordinary amœbeæ or amœboid stages in the life history of other organisms, I am unable to say. These amœboid organisms were not numerous, and were only found in some of the masses; they appeared to lie in the more internal, softer part of the substance. On an examination of the green-tinted lumps, the same general appearances were seen, but the number of unicellular Algæ was much greater, and their forms and sizes were more varied. There was also found, here and there, a single Closterium (Desmideæ); but even in these green masses, the various species of Schizomycetes vastly preponderated. The " Bacteria " were nowhere found to be motile. There were no appearances like those found in the gelatinous Algæ (Nostocacæ and Rivulariæ, etc.) The general ground substance, though very soft, evidently contained some cellulose, for it gave well the characteristic micro-chemical reactions of this substance with Iodine and Sulphuric Acid, and with Schulz' staining fluid.

The further examination was conducted by hardening some of the mass, and preparing thin sections of it, as well as by more careful examination of the fresh material. Some was hardened in strong alcohol; in bichromate of potash 1 %; and in strong picric acid; but the best results were obtained by slowly staining a lump *en masse* in weak and afterwards in strong logwood solution, and then hardening in Alcohol. When sufficiently hard, sections were cut, and stained, some in logwood, some in various aniline dyes—Spiller's purple, methyl-violet, &c. The appearances presented by careful examination of these sections were essentially the same in all cases, and are here briefly described.

The ground substance, structureless and unstained, contained, imbedded in it, the following structures:—

I.—Schizomycetes—Species of the Genera, *Bacillus*, *Bacterium*, *Vibrio*, and *Spirillum*.

(a) *Bacillus*.—The great majority of the organisms found are of this genus. They mostly appear to be of

the species *B. Subtilis* of Cohn[1] and are chiefly found singly or in twos, less frequently in threes or fours. Many of them are seen in long rows, or in the leptothrix condition. Here and there, especially near the surface, bundles of such leptothrix threads are found. The solitary Bacilli are mostly quite homogeneous rods of protoplasm, but some are seen to contain either a single spore or two spores, and several such Bacilli are, here and there, found in a long chain. There are also seen, at intervals, considerable accumulations of these spores, which may also be found in every stage of germination into Bacilli. In addition to the leptothrix *Bacillus subtilis* there are also found a few thicker leptothrix threads, which are seen to be branched in a more or less dichotomous manner. These I identify with the so-called *Cladothrix dichotoma* of Cohn. It is really the leptothrix condition of another species of Bacillus. According to Zopf[2] Cladothrix has been seen to give rise to micrococci, bacilli, bactera, and spirilla, though this is doubted by Klein[3]. When unstained, these threads appear as smooth, pale, slightly granular, branched rods.

(b) *Bacterium.*—I find considerable numbers of both the common species of Bacterium, viz.:—*B. termo* and *B. lineola* of Cohn, but chiefly the latter. These species are very similar to each other in form, but *B. lineola* are larger and longer than *B. termo*. They are both readily distinguished from *Bacillus subtilis* by being shorter relative to their breadth, and by being more rounded at their extremities. Some of them are found to be constricted in the middle, *i.e.* in the act of fission.

(c) *Vibrio.*—A few thin and long Vibriones are seen, but not many. They have a wavy appearance, usually with two or three waves, and appear to answer to Cohn's *Vibrio Serpens.*

(d) *Spirillum.*—There are many more spirilla than Vibriones. They all appear to be of the same species, viz., *S. tenue*, and occur singly, in masses, and in long

(1) *Beiträge Zur Biologie d. Pflanzen.* Vol. II., 1872.
(2) *Zur Morphologie der Spaltpflanzen*, Leipzig, 1882.
(3) *Micro-organisms and Disease.*

chains. The chain-like condition is by Cohn distinguished as a separate genus—*Spirochæta*.

II.—Algæ. All the Algæ found are unicellular; there are a few spindle-shaped ones, but the majority are simply rounded cells, the cell walls of which are distinct; some are oval; they all contain chlorophyll, which obscures the nucleus. They are chiefly Pleurococci and Protococci, and have no definite arrangement.

One might be inclined to regard the growth as of a lichenous character, *i.e.* Fungi parasitic upon Algæ, but from the immense preponderance of "Bacteria" over the Algæ, and from the extreme scantiness of the latter in most of the masses, I am rather inclined to look upon the substance as a huge mass of "Bacteria," which have produced a gelatinous material by metamorphosis of the cellulose of their cell-walls in the process of their growth; and the presence of Algæ I am inclined to regard as due to accidental entanglement of them in the gelatinous growth. If this view be correct, one must regard the growth as a huge *zooglœa*, *i.e.* a gelatinous mass formed by the swelling of the cell-walls of numbers of "Bacteria," and whose exceptional characters may have resulted from the fact that so many species of "Bacteria" are agglomerated together, and are engaged in a common "struggle for existence."

Biologists now generally classify the Algæ and the Fungi into two parallel series of forms of the large group Thallophyta, and the Schizomycetes occupy the same position in the Fungi series as the Nostocaceæ and Rivulariæ do in the Alga series. It is interesting, therefore, to find the Schizomycetes assuming an external appearance similar to that of the gelatinous Algæ (Nostoc, Rivularia, &c.)

Perhaps the most extraordinary circumstance in connexion with this material is the fact that it has appeared spontaneously in what is apparently quite fresh, clear, running spring water.

An account of the analysis of the water by Mr. Brierley follows this paper.

The spring in which this growth occurs issues from a bed in the Lower Bagshot sands. The surface of the ground rises towards the east and north-east, and forms a slope to-

wards the west and south-west. As the pool of the spring had become filled up with sand, it was dug out in the summer of 1887, and an armful of small branches of arbor vitæ was laid at the bottom of the pit thus formed to keep back the sand from which the water springs. The sides of the pit were then roughly boarded round to prevent the sand falling in, and a pool about five feet deep, and about four feet square at the top, was thus formed. The growth of this material was noticed about three months after this work was completed. The maximum flow of water at the spring is about 450 gallons daily.

Note.—Since writing the above account I have accidentally found a short note by Professor Ray Lankester, in the "Quarterly Journal of Microscopical Science," 1879, page 116, in which it is shown that the so-called "ropy change" of Beetroot Sugar manufactories is due to the action of a micrococcus. The structure of this ropy material is shown to be probably only a variety of the Ascococcus Billrothii," first described by Billroth, and afterwards by Cohn (Quart. Journ. of Micr. Science, Vol. xvi., 1876, p. 264). Similar gelatinous spherules and masses develope spontaneously on slices of cooked beetroot, when kept exposed to the air and moist. Examination of these shows apparently the same structure as that described in the present paper, but the gelatinous substance developed on cooked beet is stated by Lankester to produce no result when treated with Iodine and Sulphuric Acid. Mechanical agitation is said to favour the production of the "ropy" change. It is interesting to find that the process of jelly-formation, so frequently found in the Algæ, is also well known as one of the results of the action of Bacteria.