is necessary to work at such high current-densities that external cooling is required to control the temperature of the cell.

The preliminary investigation of the conductivity of bacteria and their behaviour in ultra-violet light was made in the Thompson-Yates Laboratories of the University of Liverpool in the summer of 1901. The author is in particular indebted to the late Sir Rubert Boyce for instruction and encouragement in the earlier work, and to Professor Hutchens, of the University of Durham College of Medicine, in the later stages.

An Investigation into the Life-history of Cladothrix dichotoma (*Cohn*).

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[Plate 8.]

The name *Cladothrix dichotoma* was first applied to this organism by Cohn in 1873. In 1875 he also founded the genus *Streptothrix* to include an organism (*S. Foersteri*) which differed from *Cladothrix* mainly in the possession of a mycelial habit. In 1887 the genus *Actinomyces* was also instituted by the same writer, to include the newly discovered *A. bovis*. Whatever may be the value of the distinction made by Cohn between *Streptothrix* and *Actinomyces*, there is no doubt whatever about the clearness of the line of separation which he set up between these genera and *Cladothrix*. Unfortunately, later writers have used the term *Cladothrix* to indicate not only the only organism belonging to the group, but also species belonging to *Streptothrix*. As examples may be mentioned the organism described by Cienkowski (3) in 1877, which he describes as having a branched mycelial habit. The same mistake was made by Winter (21) in 1884. Influenced, doubtless, by these descriptions, Macé (14) in 1884 denied the separate identity of *Streptothrix* and *Cladothrix*. In his work he describes the characteristics of *Cladothrix*, and gives, under this name, precisely those defined by Cohn as belonging to the genus *Streptothrix*. The confusion by this time had become fixed, and we find the same mistake in later writers. Thus Günther and Rullmann (10), in 1896, describe as *Cladothrix odorifera* what is obviously a *Streptothrix*. Again, Acosta and

y Grande Rossi (1) describe as *Cladothrix invulnerabilis* an organism with a branched mycelium and "aërial hyphal threads." The same indubitable characteristics of *Streptothrix* are to be found in Eppinger's (5) *Cladothrix asterioides*, and in Hesse's (2) and Garten's (9) *Cladothrix liquefaciens*, likewise in the organism described as *Cladothrix* by B. Fischer (7), Kedzior (13), Naunym (16), Tchierchke (19), and Flügge (8). Some investigators had avoided this mistake. The first, since Cohn, was Zopf (22), whose masterly treatise, despite small errors in detail, contains the best morphological account of *Cladothrix dichotoma* which has yet been published. We are also indebted to Büsgen (2) and to Hoeflich (12) for several valuable additions to our knowledge of this organism, and particularly because their observations were taken from pure artificial cultures. To Büsgen belongs the credit of being the first to obtain a pure culture of this species, while the fullness of Hoeflich's account of growth in artificial media leaves nothing to be desired. On the other hand, the researches of Sauvageau and Radais (18) have put our knowledge of the genus *Streptothrix* on a firm basis. We know now that the two groups are, phylogenetically, very far apart, and that, with one exception, *Cladothrix dichotoma* stands alone in the group Cladothricaceæ. This exception is *Sphærotilus natans*; the inclusion of this organism and *Cladothrix dichotoma* into a single group (called Sphærotilus) by Migula (15) is a step the wisdom of which is very questionable.

Distribution.—Cladothrix dichotoma was described by Zopf as the "Wasserpilz par excellence," on account of its very wide distribution. He referred more particularly to the neighbourhood of Berlin. In the neighbourhood of Glasgow the organism does not thrive particularly well. In waters with a high organic content the predominant place is held by one or more species of the lower bacteria, or one of the higher fungi. In two places only was a predominant growth of *Cladothrix dichotoma* found in waters containing organic matter in solution. From one of these, a ferruginous stream running close to Possil Marsh, near Glasgow, the whole of the present observations were taken. The water which forms this stream is collected from a neighbouring cemetery that is situated on a slight eminence. This accounts for the comparatively high amount of organic matter in solution in this water.

Analysis of the Water.—This showed that in all respects the water was of the same character as a very dilute sewage. In addition, it contained a small amount of ferrous salt in solution, and of course a large amount of the insoluble red ferric hydroxide which imparts the characteristic colour to streams of a ferruginous nature.

General Characteristics of Cladothrix dichotoma.—The organism consists

usually of long colourless threads of 1–3 μ in width. When undisturbed, the length may reach up to 3 mm. Each thread consists of a single row of rods enclosed in a delicate sheath. The thread may be free, or attached to blades of grass or other similar objects. When free, the thread may be floating or it may assume motility. Its general microscopic appearance is shown in fig. 1 (Plate 8). The rod-shaped cells vary in length, but the average is 3–5 times the width. When the thread is attached the cells at the free end are, on the whole, longer than those at the other end. In some threads the cells may be some distance apart (fig. 2). The falsely dichotomous appearance of this species (fig. 3) is seen only when the stream is moving very slowly or is at a standstill. False dichotomy is due to the growth into threads, of cells which have slipped out sideways. In my own cultures its formation was quite exceptional.

The Sheath.—The sheath forms a hollow tube, closed at both ends when the thread is young (fig. 4). In the older condition, if attached, the thread opens at the apex, when the enclosed cells escape into the surrounding water (figs. 2 and 18). It is generally assumed that the sheath is a delicate but firm textured envelope in the form of a hollow cylinder, and that this condition is maintained throughout the life of the plant. All my observations show, however, that the sheath is a clinging mucilaginous envelope during the early stages of growth and only later assumes a firm consistency. It is developed by a mucilaginous secretion of the walls of the young cells. In very young threads, composed of two or three cells only, there is no trace of a sheath. This structure can best be studied by means of iodine preparations, for the cells become deeply stained, while the sheath, though remaining uncoloured, becomes more opaque; further, the cells contract somewhat, thus making the sheath a more conspicuous object (fig. 5). In normal healthy threads, before the splitting of the sheath at the apex, *there are transverse bars of the same material as the sheath between each cell.* These can best be demonstrated by leaving freshly collected threads in ether for two or three days in order to cause the cells to contract (fig. 6). The contents of the cells will have then become disorganised and the cells themselves noticeably smaller. The same result can be produced by iodine if material at the right stage be selected (fig. 7). These bars are also often seen in old threads that have become partially disorganised (figs. 8 and 9); in such cases the cells decompose faster than the sheath. Previous writers make no mention of these transverse bars and the only indication that I can find of their existence in the works of previous writers is in fig. 17 of Büsgen's (2) publication. On p. 151 he says, "Von anderen Entwickelungsständen der Cladothrix traten in meinen Culturen nur noch eigentümlich gestaltete Faden

auf, welche sich durch besonders hormogonienbildenden Algen vergleichen lassen. Dieselben bezeichneten sich durch besonders dicke Scheiden und eine auffallende Gruppirung ihrer Stäbchen aus. Oft ebenso lang wie breit, lagen die letzteren beispielsweise in weniggliederigen Reihen zusammen, welche durch Pfropfen einer structurlosen Masse, wohl Reste abgestorbener Stäbchen, getrennt waren."

If this figure be compared with fig. 10 of this publication, in which two of these broad transverse bands are shown, it will be seen that the mapping out of the "hormogonia" was probably due in his cultures, and certainly in mine, to the formation of very broad transverse bands of the same material as the sheath. But, under appropriate treatment, these transverse bands can be found between each cell. In fig. 10 we see two of these broad bands, viz., at *a* and at *b*. They are obviously formed by the accumulation of sheath material in those spaces left empty by the slipping out of one of the cells through the sides of the sheath. The space occupied by this cell then becomes filled with the same material as that composing the sheath, and so the appearance presented in fig. 10 at *a* and at *b* is gradually formed. When the length of such a band is equal to that of one of the cells, it often has a hollow consistency, the hollow space being obviously that previously occupied by a cell. In old sheaths there is no trace of the transverse bars. This may be explained as follows: it is obvious that the sheath, at first soft, later hardens. The growth of the contained cells however continues, with the result that the apex of the sheath is burst through and the sheath, now hard, remains permanently open. Whilst the sheath is hardening, however, the pressure of the growing cells causes the latter to break through the transverse bars, and as they closely fit the sheath the movements of the growing and dividing cells would soon smooth down all projections caused by the remains of the bars; this would take place whilst the sheath was hardening and, of course, after hardening no more transverse walls would be formed. It is interesting to note that a similar secretion of mucilage, followed by a similar hardening of this material, resulting in its complete separation from the cells has been described by Hansen for yeast-cells (see Klöckers, 'Gärungsorganismen,' p. 174). In such cases the yeast-cells are enclosed inside the meshes of a hardened reticulate mucilaginous network. This hard network is obviously the same kind of material as that forming the sheath of the *Cladothrix* cells.

The Vegetative Cells.—The cells are usually 3–5 times as long as they are broad, though they are shorter in growing cells (figs. 1, 2, 4, 10). The average width is about $1\frac{1}{2}\,\mu$. By the use of stains, the cell is seen to be bounded by a sharply defined double contoured membrane. The best stain for the purpose is iodine. The membrane is coloured a deep brownish red,

the cytoplasm a light transparent brown, whilst the sheath remains uncoloured. Methylene-blue, or carbol-fuchsin, or Bismarck-brown may also be used for staining, but iodine is undoubtedly the best. In young cells the cytoplasm is unvacuolated, and in it are usually found some strongly refractive round bodies. These are oil-bodies and have already been noticed by Büsgen (2). In artificial cultures the oil-drops are wanting and in their place we find large empty spaces in the cytoplasm. Hoeflich (12), who worked with artificial cultures, does not mention them, but states that the cytoplasm is strongly vacuolated, hence we may conclude that in artificial cultures the conditions are not favourable enough to enable the organism to store food material. For their observation I have taken samples direct from nature, and examined as soon as possible after collection. This is necessary because the threads decompose very rapidly. A cell may have two or three large oil globules or a larger number of small ones (fig. 11, *b*). In many cells I also found glycogen as a reserve material, either with or without oil-globules (figs. 12, 13). This reserve material was sometimes found as a covering to the oil.

I am strongly of the opinion that the *membrane is often used as a depository for reserve food material.* This is shown by the fact that, when there was much glycogen in the cell, the colour of the membrane when stained with iodine could not be distinguished from that of the glycogen deposited in the cytoplasm. On the other hand, the membranes of cells belonging to threads of a five to six days' old culture, in which glycogen was never found, were markedly free of colour when treated with iodine. It must be borne in mind that the membranes of bacteria and their allies have not the same firm consistency as have those of the higher plants. They are more readily permeable, and solid substances enclosed in the cell can sometimes be seen being thrown out without any apparent difficulty when, during staining, the absorption of certain liquids sets up a high osmotic pressure. In the case of *Spirillum volutans* I have on several occasions observed the ejection of volutin granules during the process of staining. Such membranes are probably well adapted to serve as depositories of food material, and it is a membrane of this kind that *Cladothrix* possesses.

Some interesting facts are brought to light by the observation of threads that are gradually undergoing decomposition. Large gaps are seen in the cytoplasm, marking the spots originally occupied by the reserve food material; the membrane, being more resistant, stands out clearly in such cells, thus giving us perhaps the best evidence of the morphological unity of this organ (fig. 15). In still older threads the individuality of the cells is lost, and finally the whole breaks down, when irregular lumps of

cytoplasm may subsequently be seen in the surrounding fluid. These lumps must serve as a valuable source of food to the other micro-organisms in the water.

Hoeflich (12) in p. 61 speaks of vacuoles as though they were obvious constituents of the cell; as he does not mention the presence of oil and worked with artificial cultures, I think it probable that his vacuoles were either oil drops or empty cavities in the cytoplasm. On several occasions I have observed apparent vacuoles in the stained cells of artificial cultures, but as these might just as well be referred to the spaces originally occupied by the food material I have refrained from drawing any conclusions from the fact.

False Dichotomy.—The characteristic tree-like appearance of *Cladothrix* (fig. 3) is assumed only when the water is practically stationary. A cell slips out sideways through the mucilaginous sheath and grows into a thread, the mucilage holds the branch and parent together, and so it appears as if true branching had taken place. In fig. 16 is shown a "branch" attached to the parent thread. At the point of attachment there were no indications of the existence of two separate sheaths. As the cells which give rise to the daughter-thread slip out through a very soft sheath, such is not to be expected, and when later the mucilaginous sheath hardens, at the point of contact of the daughter- with the parent-thread, the sheath would harden as one piece. When the conditions are not restful, the falsely dichotomous growth is not possible, for the mucilaginous sheath has not sufficient adhesive power to prevent the escape of the liberated cell, but, if the conditions are restful, adhesion is possible, and, when the sheath later hardens, the escape of the daughter-threads is rendered quite impossible, even if the water becomes subsequently somewhat less restful. It is, therefore, only under exceptional conditions that the *Cladothrix* of the text-books is formed.

Cell-division.—The stages of cell-division can be followed by reference to fig. 15. There is first a slight elongation of the cell, then a slight constriction in or near the middle, after which a thin transverse membrane is thrown across the cell at the constricted part. The constriction gradually deepens and the membrane becomes thicker; this is followed by a drawing apart of the daughter-cells, after which each elongates until the adult form is reached. The process is thus essentially the same as in the Bacteriaceæ Stained preparations sometimes show that the separated cells are connected by a mucilaginous thread (fig. 17). No doubt, for a time, a thin thread of protoplasm, running through the mucilage, also joins the cells.

When the sheath hardens, the pressure and growth of the dividing cells causes a rupture of the sheath at the apex, after which the sheath remains

permanently open. This pressure also causes the side-slipping of individual cells before the sheath hardens, resulting, if the water be moving very slowly, in the cell growing into a "false branch."

Cell-division takes place at first uniformly throughout the thread, but, after the apex has been opened, it is mostly confined to the basal part, as at the apex only full-developed mature cells can be observed.

Multiplication.—This is essentially of one kind, viz., by the rejuvenescence of a single cell or a group of cells. The following varieties of this mode of multiplication may be distinguished :—

1. *Multiplication by Liberation of Thread-fragments.*—This is the mode followed by motile threads, in which I have seen no trace of a ruptured sheath nor of the liberation of single cells. Some of these motile threads may reach to a length of $1–1\frac{1}{2}\ \mu$, and even in these the sheath is soft, and envelops the cells very closely. The liberation of thread-fragments has been observed by Zopf in the case of attached threads, and I am able to confirm his statements. In my observations, however, the liberated fragments assumed a spiral form. I propose dealing more fully with this in a later section.

2. *Multiplication by the Liberation of Rejuvenated Single Cells.*—On the nature of this mode of multiplication all observers are agreed. Sooner or later, in attached threads, the apex of the sheath bursts, and single cells are pushed out into the surrounding water. These cells are sometimes devoid of motility and drift placidly away, at other times they develop organs of motion, by the aid of which they swim out of the sheath. I have observed the actual liberation of a non-motile cell. It seemed to be thrust out of the sheath by the aid of a small push from behind (fig. 18). There is nothing of a sporogenous character about these cells, for they behave in precisely the same way, as regards growth, division, etc., whether within or without the sheath. They are vegetative cells of the same nature as those of the genus *Bacillus*. Hoeflich follows this line of thought so far as the non-motile cells are concerned, but he refers to the same when motile as "spores," because, previous to ejection, in addition to the development of cilia, certain changes in vacuolisation occur. The changes which he describes seem to me to be connected with changes incident to the development of cilia. The only difference, therefore, between what is confessedly a vegetative cell and what he calls a "spore" is that the latter is motile. These "spores" appear to me to be merely swarming vegetative cells. Hoeflich does, indeed, refer to the germination of these bodies, but, considering the importance of the point, a bare mention of the fact, and a very inconclusive drawing, are not sufficient evidence to warrant full acceptance without confirmation.

The Formation of Spiral Threads.—Since the announcement of Zopf in 1882 of the fact that under certain circumstances *Cladothrix dichotoma* liberates spiral threads which behave like *Spirilla*, criticism on this point has been hostile. Winogradsky (20) was strongly of opinion that the life-cycle of *Cladothrix dichotoma* was completed by the development of "swarming red cells" which reproduced the threads. He professed himself very sceptical as to the formation of spiral reproductive cells, though pointing out that wavy threads were not uncommon in this species, which every investigator of this species has of course observed.

Following Winogradsky, the opinion of all subsequent researchers on this subject has set in the same direction. Büsgen (2) accounts for Zopf's *Spirillum* threads by supposing that a *Spirillum* species was present as an impurity, and this in spite of the fact that Zopf had mentioned (p. 10 of his 'Zur Morphologie der Spaltpflanzen') that he had actually observed these spiral fragments being detached from the thread. Hoeflich (12) takes up the same attitude and denies the existence of spiral threads. The consequence has been that modern writers of text-books on this subject have omitted all mention of these *Spirilla*. Being convinced of the truth of Zopf's observations on this point I have examined hundreds of samples of *Cladothrix* cultures extending over a period of 18 months, in order to be able to confirm his observations.

How the conditions arose I cannot say, but in one culture, and one only I observed the whole procedure as outlined by Zopf. This was in a seven-day-old culture in an open beaker. The medium consisted of a solution of ferrous carbonate to which a drop of 0·05-per-cent. peptone had been added. Between the 7th and the 10th day, the organism fell to pieces. The culture was impure, it is true, but *Cladothrix* threads are unmistakeable after a very little experience. On the 7th day I found the tufts of threads in a state of violent trembling. The surrounding water contained spiral threads that in the unstained condition could not be distinguished from typical *Spirilla.* Cilia preparations showed these to possess polar ciliation. Each was seen to be a *Cladothrix* thread of three to five cells, and with polar cilia. The trembling of the tuft of threads was obviously due to the violent wriggling of these spiral forms in their efforts to free themselves from the colony. Examples of these are given in figs. 20 to 24. Each *Spirillum* had 1 to 3 polar cilia. The *Spirillum* consisted of from half to three wave-lengths. Some consisted of one cell only. *The cilia preparations showed spiral fragments in all stages of liberation.* In fig. 25 is shown one that has been newly liberated. It was not possible to ascertain whether these *Spirilla* permanently retained the spiral form or whether they settled on some object

and developed into typical *Cladothrix* threads: probably both sequences followed. Winogradsky, writing on this point, says: "Nur dann konnte man den genetischen Zusammenhang eines Spirillum mit Cladothrix für bewiesen halten, wenn er einen Cladothrix-faden aus einem echten noch gewissermassen unter Beibehaltung seiner characteristischen Eigenschaften sich teilenden Spirillum erzogen hätte." It is proved at any rate that spiral forms with spiral movements and polar cilia are liberated from what was undoubtedly a *Cladothrix* colony. These appear in the surrounding water as organisms in no respect different from what are commonly regarded as *Spirilla*. Their subsequent fate is immaterial to the point at issue, so long as it is proved that they originated from a *Cladothrix* thread. One cannot doubt that sooner or later they settle down and develop into the typical threads. This point has not been proved. If this proof were furnished it would be an interesting addition to our knowledge of this organism, but would have only a slight bearing on the point under discussion: the essential point lies in the proof of the existence of *Spirilla* originating from a *Cladothrix* thread, not in proving what becomes of them after they have been formed.

The Formation of Cocci.—According to Zopf, *Cladothrix* also multiplies by a splitting up of its cells into cocci. I have not observed this method of multiplication. It is common in the case of *Crenothrix polyspora* when growing under unusually favourable circumstances (see Garrett, 'Public Health,' No. 1, 1896), and one must bear in mind that when during active growth the elongation of bacterial cells does not keep pace with their division, the length of the cells in such a culture is not greater than their breadth, and consequently cocci-like rods are formed. It is possible that Zopf may have observed such a phenomenon. As young *Crenothrix* threads resemble *Cladothrix* threads, it is also possible that he may have mistaken the former for the latter. The matter must be regarded as not yet settled.

Synopsis of Methods of Reproduction.—We may, therefore, enumerate the following kinds of reproductive organs as occurring in *Cladothrix dichotoma*:—

a. Rod-shaped swarm *cells*.
b. Spirally shaped swarm *cells*.
c. Rod-shaped swarm *fragments*.
d. Spirally shaped swarm *fragments*.
e. Rod-shaped non-motile *fragments*.

Artificial Cultures.—Büsgen was the first to obtain artificial cultures. He was followed by Hoeflich (12), who gave a very complete and accurate account of the growths of this organism in various media. My own

observations on this point were chiefly of a confirmatory nature. In all media growth commences by the formation in the liquid of characteristic grey, easily visible flecks. Microscopically examined, each fleck is seen as represented in fig. 19. This is, I think, the first time that a figure of a *Cladothrix* colony has been published. The presence of a large number of flecks gives the liquid a very turbid appearance. They usually congregate at the top, but fall to the bottom when gently shaken, and remain there. After a few days a flocculent grey deposit is formed. The organism is strongly aërobic, and gradually liquefies gelatine when grown in gelatine nutrient media.

Motility and Organs of Movement.—Hitherto, investigations of the organs of movement of *Cladothrix* have been confined to the cilia of single independent cells. The disposition of cilia in motile threads consisting of more than one cell has not been touched upon. Motile threads of *Cladothrix* are, however, common, and if motile single cells develop into motile threads consisting of many cells, it is obvious that a development of fresh cilia must also take place in order to secure an adequate propelling force. Cilia preparations were first made of single motile cells. The cilia of such cells have been investigated by Hoeflich (12) and by A. Fischer (6). They were shown to be situated in a sub-polar position. Unfortunately, I could not obtain cells in a swarming condition inside the sheath, but I was able to obtain cilia preparations of several immediately after their liberation from the sheath.

In all such preparations the cilia were disposed in the polar and not in a sub-polar position (figs. 26, 27). With regard to the cilia of motile threads, in these also the cilia were placed at the poles of the individual cells. The cilia of a thread of seven cells are shown in fig. 28, of five cells in fig. 20, and of two cells in fig. 27. As the threads become longer the movement gets slower; threads of ½ mm. in length move with a very slow stately movement. Beyond this length movement ceases, evidently because the development of cilia has not been able to keep pace with the increase of mass caused by the growth and development of the cells.

The motility and organs of movement of the liberated motile spiral threads have already been described.

The Organ of Attachment of Attached Threads.—No indication was seen of a special organ of attachment. Büsgen mentions the existence of a small adhesive disc, but in my cultures when attached threads were liberated there was nothing to show which end had been previously attached.

The Phylogenetic Position of Cladothrix dichotoma.—The organism that is undoubtedly most closely allied to *Cladothrix* is *Sphærotilus natans*. The

latter differs from the former only in having a very delicate and slimy sheath, and in that a common sheath acts as a covering for a large number of threads. Both Fischer and Migula have united *Cladothrix* and *Sphærotilus* into a single genus, to which the name *Sphærotilus* has been given. As the mucilage secretion must be largely dependent on external circumstances, it is doubtful, I think, whether it could be demonstrated that the points of difference between the two organisms were other than mere local adaptations. In the only figure of *Sphærotilus natans* that I have seen* there was nothing to indicate that the organism was other than *Cladothrix dichotoma*. The organism described in these pages differs more from the normal *Cladothrix* in essential points than does *Sphærotilus natans*. The conclusion seems justified that we are dealing with what Migula appropriately terms a "Sammel-species." *Cladothrix* is like *Bacillus coli communis*, a cluster of sub-species clustered round a central widely distributed form. Migula emphasises this point from a physiological standpoint. The existence of the organism dealt with in these pages and of *Sphærotilus natans* demonstrates the fact that from a morphological standpoint also *Cladothrix dichotoma* must be regarded as a cluster of varieties around a central dominant form.

Amongst other organisms *Crenothrix polyspora* and *Clonothrix fusca* must be regarded as being most closely allied. Their differences are not phylogenetically of great importance. Slightly further removed are *Leptothrix*, *Gallionella*, and *Spirophyllum*: they differ chiefly in forming reproductive cells by abstriction. At the present state of our knowledge it is unwise to do more than hint at the possible relationships of *Cladothrix* with the other members of the higher bacteria. The sulphur bacteria from a phylogenetic standpoint form a highly unnatural group, and *Cladothrix* is much more closely related to forms like *Beggiatoa* than this is to the other sulphur bacteria, *e.g.*, *Thiophysa* or *Chromatium*.

The relation of *Cladothrix* to *Streptothrix*, using this term as defined by Cohn and as followed by Rullmann in Lafar's 'Handbuch der technischen Mycologie,' is a very distant one. The whole habit of the latter plant, with its mycelial structure and mycelial mode of branching, shows its relationship with the higher fungi, whilst *Cladothrix*, on the other hand, is more nearly related to the Algæ, and, had it possessed colouring matter, there would have been no difficulty in relegating it to that class. Among the Algæ, the Cyanophyceæ is the group to which *Cladothrix* has the strongest leanings. The general resemblance to forms like *Oscillatoria*, to *Hepalosiphon*, and to *Plectonema* is sufficiently striking to arrest attention. On the other hand,

* 'Report of Royal Commission for Disposal of Sewage.'

the rod-like ciliated swarming cells, the method of cell division, the nature of its reserve material, attest the fact that we cannot place *Cladothrix* very far from the lower bacteria, particularly the genera *Bacillus* and *Pseudomonas*.

The connection with the genus *Spirillum* is also indicated by the occasional thrusting forth of spiral cells or thread-fragments in all essentials identical with those belonging to the genus *Spirillum*.

In fact we see in *Cladothrix* a very good illustration of the fact that very low down in the scale of organisation, organisms tend to approach more closely in their affinities. It is in such widely distributed organisms as this that such facts are best demonstrated.

Summary.

Distribution.—Widely distributed on the Continent, but not so in Great Britain, its place being taken by members of the higher fungi.

Occurrence.—Best growth found in ferruginous waters containing a slight organic contamination.

General Characteristics.—Long, colourless threads, 1–3 mm. long. Threads may be free or attached, motile or non-motile. Tree-like colonies, exhibiting false dichotomy, formed only when the water is almost or entirely motionless.

Sheath.—Cells are enclosed in a sheath, which is soft and mucilaginous in the young condition, but later hardens; it is ultimately split at the apex by the growth of the enclosed cells, after which it remains permanently open. The sheaths of motile threads do not harden, and consequently always envelop the cells as a closely fitting sheath. *Between the individual cells, transverse bars of the same material as the sheath are formed.* When the sheath hardens, the transverse bars are destroyed by the pressure of the growing cells.

The Vegetative Cells.—The cells are arranged in a single row inside the sheath, and are $1\text{–}1\frac{1}{2}\,\mu$ in width, and, on the average, $4\text{–}6\,\mu$ in length. The cell has a distinct membrane, inside which cytoplasm and reserve matter, in the form of oil globules and glycogen, may be distinguished. Small, clearer spaces in the cytoplasm may also be distinguished, but their nature is regarded as doubtful, as they might either be vacuoles or empty spaces left after the removal of the reserve material.

Cell-division.—Is of the same nature as the same process in the orders Bacteriacæ and Coccaceæ; a transverse wall is thrown across, being followed by constriction at the same point; the constriction extends until the two daughter-cells are separated. Cell-division in the attached threads is more active near the base than at the apex, but, in the case of motile threads, is

uniformly distributed. As cell-division takes place only in one direction of of space, and transversely to the long axis of the thread, pressure is generated along the long axis of the thread, with the result that the sheath splits open at the apex.

Multiplication.—Entirely limited to the rejuvenescence of single cells or thread-fragments, which become detached and grow into new plants. No sexual or asexual spores are formed. In the case of motile threads, fragments are cut off, which grow into new threads; the same process takes place in the attached threads, the fragments being usually straight, but occasionally spiral in form. Such fragments contain usually from one to seven cells. Another variation of the same method in the case of attached threads is by the liberation, through the open apex of the sheath, of single, either motile or non-motile, cells. These cells grow into new threads. In the species under investigation, both the independent motile cells and the cells of the motile thread-fragments possessed polar cilia.

Spiral Threads.—Under certain conditions of growth *Cladothrix* liberates single cells or fragments of threads, composed of several cells, which immediately on liberation assume a spiral form, move in an undulating manner, and develop polar cilia.

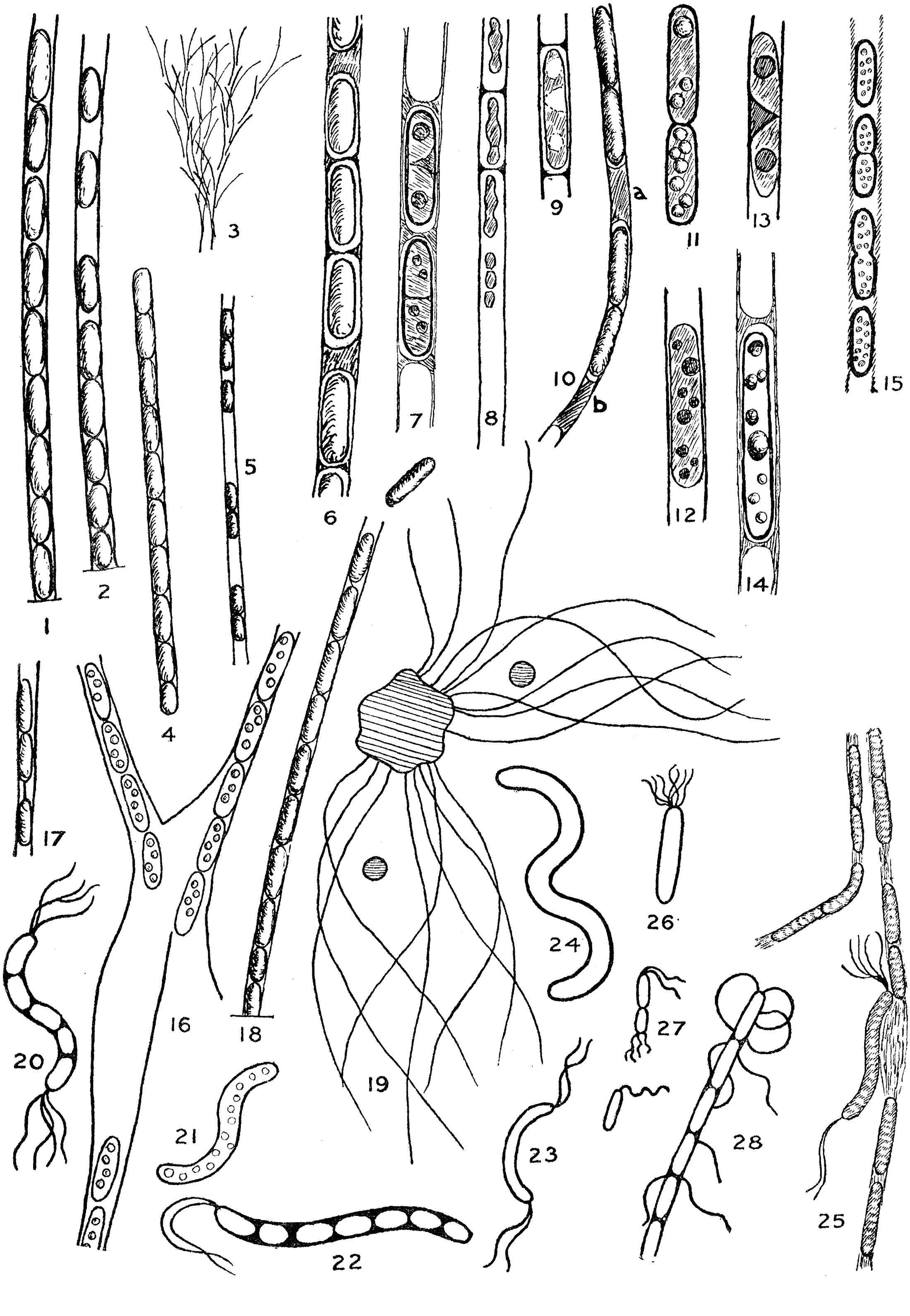
Formation of Cocci.—In the author's cultures such formations were not observed.

Artificial Cultures.—The artificial cultures instituted for confirmatory purposes showed the accuracy of Hoeflich's observations.

Motility and Organs of Movement.—The cells of the variety investigated by the author, when motile, in all cases owed their motility to cilia that were situated in a polar position, thus differing from the Continental varieties, in which the cilia are placed in a sub-polar position. Straight motile threads possessed one to three cilia at the poles of each cell composing the thread. Spiral motile threads, on the other hand, possessed one to three cilia only at the ends of the whole thread, the intermediate cells being devoid of cilia.

Organs of Attachment.—So far as could be observed the attached threads were held only by their own mucilaginous secretions.

Phylogenetic Position of Cladothrix dichotoma.—The most closely allied organism is *Sphærotilus natans*, the connection of which is so close that it must be regarded as a variety of *Cladothrix*. The investigation shows that morphological as well as physiological varieties of this widely distributed organism exist. The relationship to *Streptothrix* is an extremely distant one; in habits, methods of reproduction, and structure, *Cladothrix* is closely related both to the Cyanophyceæ and to the lower bacteria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EXPLANATION OF PLATE.

Figs. 1, 2.—*Cladothrix* cells enclosed in hardened mucilaginous sheath. × 2000.

Fig. 3.—Colony showing false dichotomy. × 125.

„ 4.—*Cladothrix* threads enclosed in unhardened mucilage. × 500.

„ 5.—Thread with hardened ruptured mucilage and from which most of the cells have escaped. × 1000.

„ 6.—Portion of thread containing transverse bars of hardened mucilage. × 3000.

„ 7.—A portion of thread stained with iodine, showing transverse bars and glycogen reserve material. × 3000.

„ 8.—Old threads, showing transverse bars of mucilage, and disorganised cells. × 3000.

„ 9.—One cell, enclosed in hardened mucilaginous sheath, showing gaps in cytoplasm, originally occupied by food material. × 2000.

„ 10.—Sheath showing at *a* and *b* broad transverse bars of mucilage. The mucilage has filled up spaces originally occupied by cells. × 1500.

„ 11.—Two cells showing reserve material in form of oil drops. × 3000.

„ 12, 13.—Cells, stained with iodine, showing reserve food in form of glycogen. × 3000.

„ 14.—Portion of thread containing one cell, stained with iodine, and showing reserve food in the form of oil drops and glycogen. The glycogen is superimposed on the oil drops. × 3000.

„ 15.—Old thread, the cytoplasm of which has almost entirely disappeared. Cell walls are less completely disorganised. Well adapted to show stages of cell-division. × 2000.

„ 16.—Somewhat diagrammatic representation of parent-thread to which a daughter-thread is attached. × 3000.

„ 17.—Portion of thread showing two cells, derived by division of a single cell, still connected by a band of mucilage, though a third of their length apart. × 1000.

„ 18.—Thread from which the cells are in process of being liberated through the ruptured apex. × 1000.

„ 19.—Colony of *Cladothrix* threads in artificial nutrient solution. Whole forms a minute gray speck, visible to the naked eye, floating near the surface of the liquid. × 100.

„ 20—22.—Spiral fragments of *Cladothrix*, showing polar ciliation. The spiral thread in fig. 20 is composed of five cells, in fig. 21 of one cell, and in fig. 22 of seven cells. × 1500.

„ 23.—Spirally twisted cell with polar ciliation, liberated laterally from a *Cladothrix* thread. × 1000.

„ 24.—A spiral fragment liberated sideways from a *Cladothrix* thread. × 1500.

„ 25.—Cilia preparation of threads from an artificial culture. Threads were in a state of violent trembling as a result of the liberation of spirally twisted and polar ciliated threads. At *a* is shown a cell on point of liberation, which has elongated slightly, become spirally twisted, and polar-wise ciliated after liberated from thread. × 1000.

„ 26.—Liberated cell, showing a comparatively large number of polar cilia. × 1000.

„ 27.—Newly liberated motile straight rod cells. × 1000.

„ 28.—Newly liberated straight thread fragment. The cells of which it is composed possess polar cilia. × 1000.

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