

IRON BACTERIA

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WITH 45 ILLUSTRATIONS AND 5 PLATES

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PREFACE

IT is nowadays increasingly felt that there should be greater co-ordination between the work of the biologist and the application of this work by engineers and chemists. This work has two ends in view. The first is to bring forward for consideration the present state of knowledge, and bring within the compass of a small book the main contributions from this and other countries. This, it is hoped, will facilitate the work of future investigators and indicate where the gaps in our knowledge are greatest. The present writer has been engaged on the investigation of the iron-bacteria for a period of ten years and is well aware of the many points which still need patient research for their elucidation. While most of the material in the book has appeared at different times in various journals, a good part appears now for the first time. It is hoped that the present volume will attract the notice of biological investigators, and induce one here and there to take up the study of the interesting group of organisms included under the iron-bacteria. To such the book will serve as a

point of concentration from which to launch further attacks. Most biologists would probably confess to a very scanty knowledge of these organisms, partly because they are not adequately treated in most text-books on biology, and partly because for efficient knowledge of them a special training in bacteriology must be superposed on a training along general biological lines. To any such, therefore, who desire a more extended knowledge of this particular class of organisms than is possible in a general text-book, this book should prove of some help.

The second end in view is to reach the notice of the water engineer and the analytical chemist, and perhaps to elicit a measure of sympathy for the endeavours of the biologist. The work of the writer brings him constantly into contact with practical problems of applied biology. There is still a lingering doubt in the mind of the "practical man" that science and practice are antagonistic, and in the work of the past, in so far as this touches the iron-bacteria, the outcome has not been the best that was possible, because problems have been attacked divorced from biological considerations. This has been especially the case in this country, and in the past we have lagged behind other countries in our appreciation of the work of the laboratory. It seems clear, for example, that certain effects due to the activities of organisms like the iron-bacteria cannot be adequately

studied apart from the study of the organisms themselves.

The practical aim of the book is, therefore, chiefly to give indications to the engineer and to the chemist of the kind of organism that they are dealing with in certain cases, and to point out the principles which should underlie the method of treatment to remove the particular evil which has attacked their reservoir or their conduit pipes. Hitherto the sources of information at the disposal of our engineers and chemists have been very scanty, and these are chiefly scattered in various journals, of which very few are written in English. The photomicrographs are sufficiently clear to ensure identification of any of the iron-bacteria if a high-powered microscope be employed. An endeavour has been made to photograph the organisms in the most typical conditions, and when most suitable for purposes of identification.

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INTRODUCTION

THE capacity for abstracting iron from the waters in which they live and collecting it in the form of ferric hydroxide on their surfaces is possessed by various representatives of most of the classes of micro-organisms that inhabit fresh waters. This capacity is a feature of certain protozoa, of certain algæ, and of certain thread-fungi. It is also characteristic of certain representatives of the large class of organisms denominated under the term bacteria. It is to such that the term iron-bacteria is applied. In the case of these organisms, the phenomenon is more marked because they not only collect more assiduously but also have excellent structures for storing the ultimate product, namely, ferric hydroxide, in the mucilaginous sheaths which surround their bodies. It is to the ferric hydroxide stored on and in the dead membranes of iron-bacteria that in most cases is due the familiar ochre-beds of ferruginous pools and streams. The deposition on each membrane is often so great that it exceeds the volume of the organism itself. Further, the membrane is preserved from the dissolution which

b

awaits the rest of the organism, and persists unchanged for many generations. A similar kind of preservation is seen in the case of various organic and other objects in the neighbourhood of certain mineral springs, with this difference, however, that in the case of the iron-bacteria the substance which accomplishes the preservation is supplied from within and not from without.

From the biological standpoint the study of the iron-bacteria is one of great interest because most of the organisms comprised in this class exhibit a somewhat higher phase of development than is met with in the vast majority of the bacteria, as they appear to have taken the first step towards what may be called the communal life. Again, they offer instructive objects for the study of a process which has a deep physiological importance, namely, the reason for this extraordinary accumulation of iron on their bodies. On the systematic side the group has little interest. It is clear that close affinity cannot be expected from organisms grouped together on account of the common possession of a peculiar physiological characteristic, a characteristic which is shared by representatives from other groups. It is true that one sub-group may be found with *Cladothrix dichotoma* as its centre, and another which centres on *Leptothrix ochracea*, but such grouping is not in any way related to the absorption of ferruginous compounds. The heterogeneous

nature of the organisms constituting this group should make us beware of attempting, as some authors have done, a primary classification of bacteria based on physiological grounds. Confusion is certain to result from attempts to substitute an artificial in place of a natural classification.

From the practical standpoint, a study of the habits and peculiarities of the iron-bacteria is one which cannot be ignored by those engineers and chemists whose work lies in the supervision of water-reservoirs. Some of the iron-bacteria occasionally multiply to an extraordinary degree in the course of a few weeks and altogether change the character of the water in which this multiplication takes place. Others again multiply in attachment to iron pipes and accelerate the formation of incrustations, necessitating the installation of new pipes or a thorough cleaning of the affected ones. In other ways also they show characteristics which make it incumbent to know at any rate the leading features in their life histories, and also the general principles which should guide all plans for the minimising of their activities.

IRON BACTERIA

CHAPTER I

LEPTOTHRIX OCHRACEA (*Kützing*)

Syn. CHLAMYDOTHRIX OCHRACEA (*Migula*)

THIS is by far the most widely distributed of all the iron-bacteria. The ochre-coloured deposit forming the beds of ferruginous streams which are so common in some districts in this country, is in almost all cases caused by the deposition of ferric hydroxide on the dead bodies of *Leptothrix ochracea*. This organism has been found in ferruginous streams in every country in which search has been made for it. Molisch (2) records that in many moorland meadows in *Austria* and in *Hungary* the water appears to be full of an ochre-coloured mass which is made up entirely of *Leptothrix* threads. The same author records that the colour of the curative ferruginous waters in Kitzbükkel (Tyrol) is due entirely to a deposit of ferric hydroxide on countless threads of *Leptothrix ochracea*. The water of the river Moldau in its course through S.W. Bohemia before it joins the Elbe, flows through long stretches of moorland country

and assumes a brownish colour in consequence of the large amount of organic matter which it takes up in solution. In this brownish water, both in the Moldau and the Elbe, Molisch has found active growths of *Leptothrix ochracea* in company with other iron-bacteria. Investigators in *Belgium* record the same wide distribution in that country. Thus Schwerts (1) has examined the deposits of 208 iron waters and has found *Leptothrix* to be the dominant organism in 142 of the samples. In Great Britain also *Leptothrix ochracea* has an equally wide distribution. In the West of Scotland, and particularly in the S.W. portion, ferruginous streams with ochre-coloured deposits are extremely common, and in practically all of them *Leptothrix ochracea* is the dominant organism. I have examined samples not only from various parts of Great Britain, but also from Russia, Spain, and Denmark. Altogether some 300 samples of the deposits of ferruginous streams were examined. In 90 per cent of them *Leptothrix ochracea* was present, very often to the practical exclusion of all other organisms. All the samples were drawn from the deposits of running or stagnant waters of a ferruginous nature. Finally Molisch records that iron-waters were examined by him in Ceylon, Java, China, Japan, and America (Chicago), and that the contents of these were in nowise different from those of the European ferruginous streams.

This organism is therefore universally distributed in ferruginous waters in every country throughout the

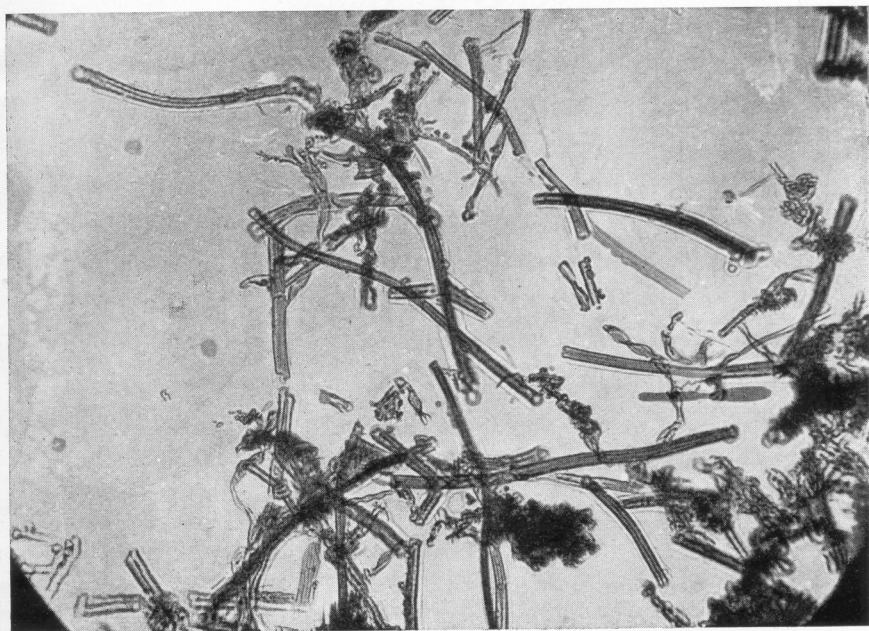


FIG. 1. $\times 350$



FIG. 2. $\times 350$

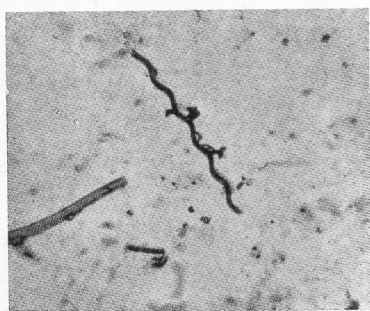


FIG. 3. $\times 350$

LEPTOTHRIX OCHRACEA

FIG. 1. APPEARANCE TYPICAL OF OCHRE-BED DEPOSIT WHEN VIEWED THROUGH MICROSCOPE. THE STRAIGHT OR SOMEWHAT BENT THREADS ARE LEPTOTHRIX. IN THE FIELD ARE ALSO A FEW BANDS—THOSE SHOWING SPIRAL TWISTING—OF SPIROPLHYLLUM. ALL THE ORGANISMS ARE DEAD, AND THE STRUCTURES ARE THEIR IRON-ENCUSTED REMAINS

FIG. 2. SPECIMEN SHOWING SLIGHT CURVATURE

FIG. 3. SPECIMEN SHOWING THE BODY THROWN INTO A NUMBER OF REGULAR WAVE LENGTHS

world. No records appear of its presence in non-ferruginous waters, but we must not conclude that it is necessarily confined to iron-containing waters, for, as will be shown later, *Leptothrix* can be cultivated artificially in a medium totally devoid of iron. Hitherto, however, in nature, it has not been found in waters devoid of iron in solution.

Nomenclature.—This organism appears under three names :—

1. *Leptothrix ochracea*.—Bestowed on it by Kützing (1) in 1843. *Leptothrix* was an algal genus, and as Kützing imagined that the species discovered by him was an Alga, it was placed in the genus in which it seemed to have the nearest relatives.

2. *Cladothrix dichotoma*.—In Zopf's "Zur Morphologie der Spaltpflanzen" (1882) organisms which were indubitably *Leptothrix ochracea* were described by this writer under the name *Cladothrix dichotoma* owing to a false impression as to their identity.

3. *Chlamydothrix ochracea*.—Migula has thought fit to bring *Leptothrix ochracea* into the fold encompassed by his genus *Chlamydothrix*. As will be shown below the change of nomenclature is open to challenge and there are weightier reasons for retaining the old generic name.

Structure.—With the aid of a good microscope the threads of *Leptothrix* can readily be identified. A photomicrograph of the organism is shown in Plate I, Fig. 1. This was taken from the deposit of a ferruginous stream. As to the deposits it must be

borne in mind that they are not composed of *living* representatives of the organism; the observer sees only the empty tubular, iron-encrusted sheaths of the dead organism. These are readily identified as the remains of *Leptothrix ochracea*, the yellow-red, sharply defined sheath being of a very distinctive nature. Whilst the majority of the threads are straight, some show slight curvatures (Plate I, Fig. 2). Occasionally, as seen in Plate I, Fig. 3, the thread is thrown into a number of undulations of regular wave-length. In the deposits the threads that come under observation are very seldom whole organisms. They are rather fragments, as can be observed from an examination of their ends, which appear as if they had been snapped across. This would doubtless happen during the process of deposition and penetration of the ferric hydroxide.

The appearance of the living organism is very characteristic. The thread is tubular, delimited externally by a delicate membrane which is normally much thinner than the dead membranes as found in the deposits. The ends are rounded off symmetrically. When grown in artificial cultures devoid of iron the membrane remains thin and delicate (Plate I, Fig. 3), and even in nature this thinness and delicacy is observable. Usually, however, the membrane increases somewhat in thickness and assumes the familiar ochre-coloured appearance. The average threads measure $1\frac{1}{2}\mu$ to 2μ in thickness when free from an external deposit of iron. When dead and caked with

iron the thickness may extend to 3μ and more. The length of the threads may reach to 200μ , and even greater lengths have been observed. In artificial cultures (Plate I, Fig. 4) they sometimes elongate considerably and assume an appearance markedly different to that found in nature. The threads increase in length, but whether the growth is intercalary or terminal has not yet been determined; on *à priori* grounds the former is the more probable.

There is concurrently a very slight increase in thickness; this, however, must be very small and must cease long before the extension in length has been completed, for long and short threads are not appreciably different in thickness, although both are appreciably thicker than very young threads.

Of the internal structure of *Leptothrix* we know very little. By the ordinary methods of staining only a uniform homogeneous structure can be observed within the threads. As is the case in the vast majority of bacteria, the threads show no distinction of cytoplasm, nucleus, and vacuoles. Cell-division by fission is a common occurrence in growing threads. Whilst in some both ends are rounded off, in others only one end is so formed, the other end being angular as shown in Fig. 1. In others again both ends are angular (Fig. 2). Until the first division takes place a young thread is invariably rounded at both ends. The reason for this failure of the cut threads to round off the end at which division has taken place is probably connected with the growing

rigidity of the membrane at this stage, as the iron deposition is by this time comparatively far advanced. A transverse membrane is never formed, the organism differing in this respect from the genus *Bacillus*. The

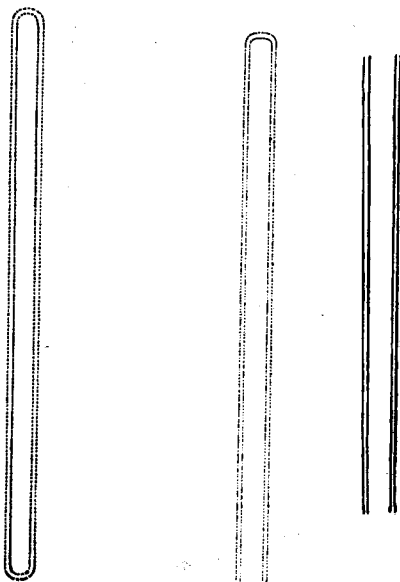


FIG. 1.

FIG. 1a.

FIG. 2.

FIG. 1.—*Leptothrix ochracea*. Figure from Molisch's "*Eisen-bakterien*".

FIG. 1a.—*Leptothrix ochracea*. Thread is rounded at one end, square cut at the other.

FIG. 2.—*Leptothrix ochracea*. Thread square cut at both ends.

details of the operation of division have not yet been satisfactorily worked out. In some cases I have observed the formation of thickened rings of the form shown in Fig. 3, at the point of division, and it would appear that fission takes place between the rings. It is curious that the rings should often be

placed not transversely but obliquely to the membrane, and this accounts for the fact that in many of the older threads the surface of the ends is not set at right angles to the sides, but is obliquely placed. The shape of the thread under the circumstances is not unlike that of a quill tooth-pick. For the observation of living threads search must be made in the stream itself and not on the bed. Fluffy bits of floating or immersed material must be sought for, and these are best found in early summer or late autumn. Apparently at these times the constitution of the organic matter in the stream best favours the growth of the iron-bacteria. Threads that are covered with ferric hydroxide should be treated with dilute hydrochloric acid to get rid of this deposit. When this is done the membrane will be uncovered and will be found generally to be of a slightly yellow-red tinge and sharply delimited (Plate I, Fig. 1). It often happens that on treatment with hydrochloric acid the deposit peels off in segments, in which case the membrane is laid bare in some parts of the thread, whilst in the other parts the membrane is overlaid by the deposit (Fig. 4). In such cases it is possible to ascertain the fact that the deposit of iron in the old threads is *inside* as well as outside the membrane, so that the ferric hydroxide is actually inside the cell in the older threads, a fact which we must bear in mind when dealing with the physiology of the iron-bacteria.

Another point to observe in the structure of *Leptothrix* is that, in common with practically all low

organisms of this grade, the real membrane is invested by a very tenuous, mucilaginous, under ordinary circumstances invisible mantle. This envelops the membrane and covers its surface much in the same



FIG. 3.



FIG. 4.



FIG. 5.

FIG. 3.—*Leptothrix ochracea*. Showing thickened rings between which division appears to take place. (Diagrammatic.)

FIG. 4.—*Leptothrix ochracea*. Portion of old thread, showing membrane from which the ferric hydroxide has been removed in parts: shows relationship between thickness of membrane and thickness of deposit of ferric hydroxide. *m* = membrane from which deposit removed.

FIG. 5.—*Leptothrix ochracea*. Appearance presented by old thread laden with thick deposit of ferric hydroxide. Outside surface rugged and encrusted with foreign particles.

way that a soapy froth covers the surface of the water out of which it has been formed. The presence of this mucilaginous layer has a considerable influence in determining the final shape of the plant, for the iron compounds drawn from the water are retained in it, and undergoing solidification by oxidation, impart

solidity and opaqueness to an otherwise very tenuous and invisible structure. In the oldest iron-encrusted threads the membrane is often no longer recognisable as a separate entity, the strain on such a delicate structure having evidently become too great for its stability, with the result that it has broken at many points, and whilst the cylindrical structure is as a whole maintained, the organism appears as a roughly cylindrical mass of ferric hydroxide with a very irregular surface, often dotted with foreign particles (Fig. 5). Seen by itself, apart from its surroundings, such a mass would raise doubts as to its derivation from any organism.

According to Migula (1) *Leptothrix* is composed of a sheath within which a number of cells are lodged. This interpretation has not been confirmed by any other investigator who has studied the threads of *Leptothrix*. It is probable that Migula has mistaken threads of *Cladothrix dichotoma* for those of *Leptothrix ochracea*. The threads of the former, unless stained, appear to consist, as is shown later, each of a non-cellular tube, and it is only when treated with a reagent like iodine that the cells become evident. As young *Leptothrix* and young *Cladothrix dichotoma* threads are superficially not unlike, and as there has been no confirmation of Migula's statements on this point, the probability is great that the interpretation given above is the correct one.

The change, therefore, of the name *Leptothrix ochracea* to *Chlamydothrix ochracea*, instituted by

Migula on the strength of this supposed cellular structure of the organism, is not justified, and there seems no necessity for discarding the generic name *Leptothrix* which has been applied to this organism for the last seventy-five years.

1. *Multiplication*.—The most primitive method of multiplication, viz. one by fragmentation, is exhibited by *Leptothrix ochracea*. A portion of a thread is broken off, the two parts elongating once more after separation: each part then repeats the process so that in a short time a very large number of organisms is formed. In Molisch's artificial cultures (2), the threads were evidently often of great length (Plate I, Fig. 4). This is seldom the case in nature. Among the lower bacteria the formation of long threads is an indication of the intervention of unfavourable conditions, as the organism has apparently the strength to elongate, but not sufficient to enable it to divide. Speaking generally, in artificial cultures of bacteria short rods are found in the most favourable cultures, and long rods intervene only when conditions are unfavourable for growth. Although some long threads may be seen in natural waters, only comparatively short rods are normally encountered.

1a. According to Molisch an interesting mode of reproduction is exhibited when a colony (in an artificial plate culture) of this organism is pressed down gently with a coverslip. Numerous rods escape, develop motility, and then swim away. He does not appear to have investigated either the method of separation

or the cilia of these motile rods. The probable presence of cilia may be inferred from the motility.

The chief interest lies in the fact that the rods assume motility, for the young threads observed in natural waters are never in a motile condition, so far as they have hitherto been observed. According to the same writer the motile rods sometimes attach themselves to long threads of *Leptothrix*, giving the latter a false appearance of branching.

2. *Reproduction by the Formation of Conidia.*—This mode of reproduction was first observed by Migula (1) who states that their germination takes place on the threads. I have repeated Migula's observations and followed the various stages of development. The process begins by the formation on the thread of numerous small protuberances (Fig. 6). Each is approximately $1\mu^1$ in thickness. Elongation takes place until the protuberance is about 1.25μ in length; then constriction at the base follows, with the result that a small oval structure is cut off. In some cases the production of conidia is so prolific that the organism itself is completely buried in them. The subsequent development of the conidium is probably accomplished by its elongation and subsequent separation from the parent thread. This is rendered probable by the fact that in the case of some of them in their development the process of constriction is postponed until the length of the developing conidium is considerably greater than its thickness (Fig. 6 at *a*).

¹ μ (the Greek letter *mu*) is the unit of length universally used in Bacteriology, and = $\frac{1}{1000}$ millimetre = 0.00004 inch.

The appearance of the conidium is of a very unmistakable character, and we find similar structures in *Gallionella* and in *Spirophyllum*. It is therefore surprising to understand the attitude taken up by Molisch (2). On page 43 of his "Eisen-bakterien" we read: "Die conidien von denen Ellis spricht, habe ich nicht auffinden können . . . mir will scheinen, dass aufgelagerte Bakterien zellen eine conidienbildung vorgetäuscht haben, was ja bei einer Rohkultur nicht unmöglich wäre".



FIG. 6.



FIG. 7.

FIG. 6.—*Leptothrix ochracea*. Development of numerous small protuberances on thread. These may either be cut off when quite short—being then known as conidia—or they may develop further before abstriction takes place. The latter method of formation is indicated in the lower part of the figure.

FIG. 7.—*Leptothrix ochracea*. A further development of the same nature as is shown in Fig. 6. The protuberances have at this phase become some quill-like in appearance.

It is absurd to imagine that a *Leptothrix* thread in a natural water would be covered with bacterial cells derived from other sources than itself. If the structures in question are bacterial cells it is more reasonable to assume that they are "bacterial cells" derived from the *Leptothrix* thread; in other words, that they are of the nature of conidia. This is a far more probable explanation than the one which presupposes *Lepto-*

thrix to have drawn together "bacterial cells" from a variety of other organisms in the neighbourhood.

IDENTIFICATION OF LEPTOTHRIX OCHRACEA

The recognition of this species is easily accomplished by the microscopic examination of the deposit in ferruginous streams, when there should be no difficulty in recognising the empty ochre-coloured threads depicted in Plate I. When these have been identified the water should be searched for the living threads, and a search should also be made for slimy streamers in the water, which will also in all probability be found coloured with the ferric oxide. The living threads are normally non-motile, cylindrical, rounded at both ends, and varying in thickness from $1\frac{1}{2}\mu$ to 3μ or 4μ . Hitherto motility has not been observed in these threads in natural waters. The threads are naturally straight, but bent and often undulated forms are not very uncommon (Plate I, Fig. 2). The majority, however, show straight threads with sharply delimited membranes tinged with the ochre-coloured hydroxide of iron.

I have occasionally observed a colony of *Leptothrix* in a natural water in which large numbers of threads were held together in such a way that one organism

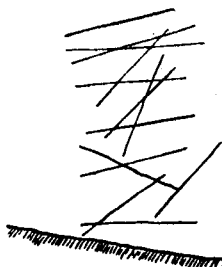


FIG. 8.—*Leptothrix ochracea*. Diagrammatic representation of a colony of *Leptothrix* threads. Threads are held together in the water by each one being in contact at one or two points with other threads.

was in contact with one or even two others at single points, as is shown diagrammatically in Fig. 8. This peculiar disposition of the threads is due to the adhesion of the threads by their enveloping mucilaginous coverings, a disposition which must occasionally happen when a number of the organisms are growing in close proximity. When, therefore, Winogradsky (1) states that *Leptothrix* branches pseudodichotomously it is probable that a somewhat similar adhesion of individuals was observed by him, which conformed to a more regular pattern, producing the effect of a dichotomous arrangement. It is certain, however, that this false dichotomy is not a *characteristic* feature of *Leptothrix*, and phenomena of this kind which are only occasional in their manifestations, are not to be relied upon for the recognition of *Leptothrix*.

LEPTOTHRIX OCHRACEA FROM THE STANDPOINT OF THE ENGINEER

Although such a cosmopolitan organism, and although present in such quantities in the waters which form the gathering grounds for the supply of many towns, this organism does not play an active part in those sudden visitations of the iron-bacteria so much dreaded by the water engineer, nor in the slower growth of the same class of organisms in the conduit pipes which furnish urban districts with their water supply. The reproductive cells of *Leptothrix* must, however, be present in all such waters, and, as

will be discussed later, the writer is of opinion that the more troublesome Gallionella and Spirophyllum are both pleomorphic phases in the life history of Leptothrix ochracea. If such be the case the comparatively harmless Leptothrix must be regarded with suspicion equalled to that bestowed on Gallionella and Spirophyllum. Therefore, it is important to the engineer to note that Leptothrix forms resting cells in the form of conidia. As shown above these minute oval structures are formed in countless numbers in ferruginous waters, and constitute the "germs" out of which new individuals arise. The question of the location of the resting cells of the iron-bacteria has often been discussed, and on a few occasions has formed the subject of an official inquiry. It may be stated that the conidia constitute, so far as is known at present, the only form of resting cells of Leptothrix. I am quite convinced that the thread itself does not as a whole become a resting cell, the so-called *arthrospore* of de Bary. This phenomenon does occur occasionally in the lower bacteria, but there is no evidence of the existence of a similar mode of reproduction in the case of Leptothrix. In all cases the threads multiply by fragmentation and then die out, but the conidia which they liberate in countless numbers persist and, under favourable circumstances, germinate to form new threads. I have not followed the germination of the conidium of Leptothrix, but have done so in the case of the conidia of Spirophyllum which are identical in practically every

respect, and the facts that have been ascertained for the conidia of one organism are applicable to those of the other. The conidia arise in water, and, until desiccation occurs, they remain in the water. As the water flows on countless numbers of these minute specks are carried along with it — it would take a million million of them to fill a small thimble—and each is potentially capable of germination when the conditions become suitable. Exceptionally they will be found in the soil in the neighbourhood of ferruginous waters, but this will happen only when the water containing the conidia has evaporated, leaving the bed dry. Under such conditions the conidia will be taken up by gusts of wind and scattered over the neighbouring soil. The following facts have been elucidated from observations of the growth of *Leptothrix* in pure artificial culture.

1. The minimum, optimum, and maximum temperatures of growth are respectively 5°C. , $23^{\circ}\text{-}25^{\circ}\text{C.}$, 40°C.

2. It grows in darkness, in diffuse daylight, and in spite of bright sunlight.

3. It grows better the greater the amount of oxygen in solution in the water.

Care must be exercised lest this organism be confused with *Cladotrix dichotoma*, for although the latter is cellular, the cells cannot be made out without careful staining, and, until stained, young threads of *Cladotrix dichotoma* can easily be mistaken for *Leptothrix ochracea*.