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XV.—*On Certain Parasites of the Mouth in Cases of Pyorrhœa.*

[*Preliminary Communication.*]

By HELEN PIXELL GOODRICH, D.Sc., and M. MOSELEY.

(Read October 18, 1916).

PLATES XII-XVII.

PYORRHŒA is such a widely distributed disease of the gums that it is hardly necessary to say much by way of introduction. To make sure, however, that the normal anatomical relations of the parts concerned are understood, it will be well to make brief reference to the microphotograph of a section taken transversely across the jaw of a puppy (figs. 2, 3).

For obvious reasons it is difficult to obtain human material suitably fixed for cytological purposes. Most of our series of

EXPLANATION OF PLATE XII.

- Fig. 1.—Diagram of a section transversely across a Mammalian jaw from the labial to the lingual side, through a single root of a tooth.
,, 2.—Microphotograph of a section through the tooth of a puppy, in the same direction as above. $\times 7$.
,, 3.—Microphotograph of the region round the gingival space of the section shown in fig. 2. $\times 35$.
,, 4.—Microphotograph of a similar portion of a section through the tooth and gum of a person with fairly advanced pyorrhœa, showing the gingival space still free from organisms. A good deal of recession has taken place; the crusta petrosa may be seen to extend nearly to the top of the figure, showing where the gum was originally attached. Note that the lesion is at the top of the gum, where clumps of *Leptothrix* and pus-cells are scattered (the tartar ridge which was attached to the tooth just above here became detached during preparation of the section). The epithelium is much infiltrated with pus-cells. $\times 50$.

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sections are from dogs or cats ; in these animals the disease runs much the same course as in man.

The normal tooth (figs. 1 and 2) has its roots deeply embedded in its socket in the bone of the jaw, and is separated from the bone by a layer of fibrous connective tissue, the periodontal membrane, and is firmly held in position by a strong ligament. This ligament is attached normally to the very top of the crista petrosa at the place where this layer of bone joins the enamel of the crown. The epithelium of the gum dips down round the tooth and reaches the ligament. Normally the gum lies closely apposed to the tooth, practically obliterating the so-called gingival space (figs. 1-3).

Even in healthy gums leucocytes from the lymph glands may be seen making their way through the epithelium, and we maintain that these glands are the main source of the salivary corpuscles always found in the mouth, and not the tonsils as stated in text-books. The corpuscles which make their way through the epithelium opposed to the tooth collect in the gingival space ("espace péri-cervical"), where Mendel (10)* has recently recorded their presence in healthy persons. We have also found them in the very long gingival spaces round the incisors of rabbits with healthy gums.

We may now pass on to the pathological changes effected by pyorrhœa. Probably the very distressing symptoms are well known to you. The clinical aspects of the disease have been much discussed and the most diverse opinions and treatments put forward, often without much scientific basis. It is from the strictly scientific side that we wish to attack the problem, and the observations given here—the result of a year's study of many hundreds of cases—are merely by way of a preliminary communication.

The symptoms in advanced cases may be summed up as—

1. Acute inflammation of the gums, accompanied by the collection of pus and a large deposit of tartar round the teeth.
2. Absorption of the edge of the socket (alveolus) and recession of the gums so that a large portion of the tooth projects, and it later becomes loose and falls out.

From a study of mild cases it is quite clear that the lesion starts near the summit of the gum (fig. 4), not at the bottom of the gingival space, as the diagrams and description given by Bass and Johns (1, pp. 55, etc.) seem to imply. This space remains for a time remarkably free from organisms. The place where the inflammation starts is directly in contact with any tartar present (fig. 5). Unfortunately, during decalcification of teeth for histological purposes, the tartar ridge nearly always becomes detached ; in the tooth shown in fig. 7, however, and on the lingual side of that shown in fig. 8, it remains more or less in position. The large

* The numbers within brackets refer to the Bibliography at end of the paper.

mass of tartar which was attached to the tooth shown in fig. 4 dropped away altogether; a section through the terminal branches of *Leptothrix* colonies from its surface is shown in fig. 14. All that is left in fig. 4 consists of loose clumps of *Leptothrix* and a good deal of pus in contact with the broken epithelium. The latter can be seen to be peeling off and to have already lost its characteristic regular appearance. At later stages (figs. 7 and 8) it disintegrates to such a great extent, and the remains become so much infiltrated with deeply staining pus-cells from the lymph glands, that it is almost unrecognizable. It is hardly necessary to point out how much the gum has receded in the figures; even in such a mild case as that represented in fig. 4 the *crusta petrosa* may be seen extending nearly to the top of the portion of dentine shown.

The peridontal layer may be seen in figs. 7 and 8 to have become very wide and necrotic-looking. It contains pus-cells and often numerous nests of nuclei which have been described as epithelial remnants. With regard to these we have not at present any conclusive evidence to offer. They are certainly sometimes present in apparently normal jaws, and, in some cases of pyorrhœa, especially in the cat, are very numerous; but we have hitherto found no micro-organisms in connexion with them, nor in fact in any part of the peridontal layer. The changes which have taken place in the bone of the jaw are apparent in both cases represented in figs. 7 and 8. The alveoli are much reduced, especially in the more advanced case (fig. 8), where the alveolus on the labial side has become quite flattened. On examining these sections with high powers numerous osteoclasts may be seen at work eating away the surface of the alveolus and also effecting lacunar absorption in all directions.

Some clinicians have objected to the name *Pyorrhœa* owing to the absence of a "flow of pus" in some cases in which recession is undoubtedly taking place. These are most interesting cases, and we are convinced that the absence of certain symptoms is only temporary and due to careful cleansing of the mouth by washes, etc. Recession in such cases is very gradual, but from continual observation of a few patients we are convinced that even here the recession is the result of inflammation. In fact our observations lead us to support the theory put forward by Znamensky (16) in 1902, that pyorrhœa always begins as an inflammation of the gums—so-called *marginal gingivitis*. Two cases of gradual recession that have come under our observation may be briefly mentioned, both in persons between thirty and forty years of age, whose mouths were thoroughly cleansed at least twice a day. In one of these the premolars and a molar on the left side showed very slight recession. From the molar the gum had receded a little more at the front than at the back, which there-

fore sloped downwards towards the front. The gum at the back of this tooth between it and the next molar was made to bleed by slight injury and then left. Next day there was a distinctly inflamed margin to the gum which appeared as a bright red rim round the back and sides of this molar. Special antiseptic precautions were then taken, i.e. mouth-washes used as often as possible. In about a week the inflammation entirely disappeared, but the gum was found to have receded a little more at the back, so that it was now nearly straight round the tooth.

In the other case the gums had receded from a canine and some other teeth sufficiently to expose the crusta petrosa, and after each short period of inflammation another narrow strip of crusta petrosa would be exposed, and this being living bone would cause great tenderness of the region for some days after the inflammation had disappeared. Thus even during a week or so of inflammation of the gum margin the alveolus appears to become slightly reduced. Possibly this may be owing to the increased number of lymphocytes in the neighbourhood, since these corpuscles are said to grow into osteoclasts and other macrophages.

Injury in such mild cases is perhaps often inflicted by the use

EXPLANATION OF PLATES XIII-XIV.

Fig. 5.—Extracted tooth, showing tartar ridge and gum flap which normally lies closely against the tooth, with the region at which it breaks in contact with the tartar. $\times 1$.

„ 6.—Extracted tooth, showing by the tartar ridge that the gum had receded unequally, leaving pockets—an especially deep one down one root. $\times 1$.

Figs. 7, 8.—Microphotographs of sections through jaws and teeth of cats with advanced pyorrhœa, showing tartar detached but nearly in its normal position. In fig. 7 a piece of vegetative tissue is jambed in on one side. The remains of the epithelium are infiltrated with the deeply staining pus-cells. The periodontal layer has become very wide. The alveoli are much reduced, especially on the labial side in fig. 8, and large lacunæ are developing in other parts of the bone of the jaw. $\times 11$.

Figs. 9-14.—*Entamoeba gingivalis*. Drawn with the aid of a camera lucida at an approximate magnification of 1500.

Fig. 9.—Living form with several lobose pseudopodia, and containing seven of the characteristic inclusions and a nucleus just discernible near the upper end.

„ 10.—Living form flattened out while feeding on bacteria, which may be seen passing inwards, enclosed in vacuoles, from the left upper side.

Figs. 11-14 from preparations stained with iron-hæmatoxylin.

Fig. 11.—Large amœba with no visible ectosarc, and the endosarc full of various food vacuoles.

„ 12.—Small form, with one large pseudopod composed of ectosarc.

„ 13.—An amœba, with two nuclei and two small lobose pseudopodia, and much food at various stages of digestion.

„ 14.—A portion of a section through a clump of tartar, showing amœbæ between the terminal branches of *Leptothrix*.

of a hard toothbrush; this might account for the fact, pointed out to us by Mr. Buxton Ryle, of Oxford, that this kind of recession is liable to be more advanced on the left side of the mouth of a right-handed person, and vice versa. Of course there are many other ways of accidentally injuring the gums which will readily occur to all. Now, we should like to suggest that if after injury to the gum the teeth are not kept free from tartar and otherwise clean, the inflammation will increase and spread until a general gingivitis is caused, and that this, if not checked, will be followed by the appearance of all other symptoms of pyorrhœa.

This is, of course, merely a suggestion, and the only evidence in its support that we can give is the very common occurrence of general gingivitis and pyorrhœa among soldiers returning from active service. They nearly all say that it was practically impossible to keep their teeth clean while in the trenches, and the condition of their gums certainly improves rapidly after the removal of the tartar by a skilful dentist and the frequent use of antiseptic mouth-washes, etc.

There is one more point to be noted among general appearances, and that is the frequent occurrence of brownish, slightly refringent granules of irregular shape deposited just below the epithelium, apparently brought there by capillaries. By reflected light this substance appears black, and was first noticed on the flaps of gum left attached to extracted teeth, which are sometimes distinctly speckled with black in consequence.

It will be well before proceeding further to give a brief account of the positions in which the tartar or calculus is found. As will be explained below, this is of organic origin, the formative organism *Leptothrix* belonging to the higher bacteria or lower plants. When its growth is not interfered with it generally forms a ridge just at the margin of the gum. After calcification to form "hard" tartar this is very firmly attached, but in its earlier stages, when with other bodies from the mouth it forms the soft tartar (or "*materia alba*" of Leuwenhoek, 1683), it may easily be removed. In an extracted tooth (fig. 5) the ridge of hard tartar is just at the top of the gum-flap which generally comes away with it, since the gum breaks at its weakest spot, viz. the lesion opposite the tartar ridge. As the gum recedes the tartar follows it, and on very neglected teeth forms a continuous encrustation, but on those that are even occasionally cleaned the upper layers are removed to a greater or less extent, so that a ridge may generally be distinguished (fig. 6). The extent to which the ridge dips down on an extracted tooth shows the depth to which the pyorrhœa pocket had extended; for example, there is clear evidence of a deep pocket having existed down one root of the tooth in fig. 6. Sometimes a pocket will extend under the crown, and here also will be found a deposit of tartar. At other times a whole root may be found to be involved.

Leptothrix has also been in two cases found growing and forming nodules of tartar at the extreme apex of the root to which there were apparently no side pockets leading. In both cases, however, the crown of the tooth had been destroyed by caries, and no doubt the Leptothrix had entered through the root canal. It may be well to mention here that we can see no reason for separating tartar into two kinds. It would be impossible to say where, e.g., the line should be drawn between so-called serumal and salivary tartars in the cases just described.

We now pass on to describe some of the parasites and their characteristic positions. The mouth swarms with all sorts of organisms—Protozoa, spirochaetes, yeasts, and especially bacteria in incredible numbers. A great deal of work has been done on these, especially the pyogenic bacteria, with a view to discovering the cause of the disease; but no particular bacterium has definitely been proved to be the cause, nor do the sections of the gum show any special invading organisms.

About two years ago a sensation was caused in the United States by the announcement by some Americans (1, 12) that the amoeba of the mouth was the specific cause; but, as we shall see, further research has not supported this view.

There is no reason to doubt that the mouth amoeba is the one described and figured by Gros (6) in 1849 from the soft tartar of the teeth, and it should therefore be called *Entamoeba* (or *Endamoeba*) *gingivalis*, Gros, as has been well explained by Smith and Barrett (12). It is quite a common parasite of the mouth; even young children may harbour it. Probably infection passes from one person to another by direct contamination, as in kissing, using the same drinking utensils, etc. We maintain that it is not to be found in healthy mouths kept scrupulously clean by the use of suitable antiseptic mouth-washes, etc. Nor have we so far found it in healthy mouths of the few animals that we have tested, such as cats, rats, rabbits, sheep and a goat.

Its favourite habitat is the tartar, and here we come into opposition with previous observers of the amoeba. It burrows, not into the tissues of the gum, as has been stated, but often between the terminal branches of the Leptothrix (fig. 14), and can generally be found in greatest numbers on the under side of the tartar ridge, where there is the greatest quantity of fresh pus. From what has been said about the position of the tartar, it will follow that sometimes the amoebæ are found in great numbers underneath the crown of the tooth, sometimes lower still, even at the apex of a root, if the pocket should extend so far. Two dogs with pyorrhœa that we have examined have shown numerous

amœbæ, but in ten or twelve cats with the disease in advanced stages we have only found them once. In both these animals the amœbæ, so far as we have been able to study them, are indistinguishable from the human one, *Entamoeba gingivalis*.

STRUCTURE OF ENTAMOEBA GINGIVALIS, GROS.

There is great variation in size among the ordinary vegetative forms. Sometimes chiefly small ones, measuring up to 10 or 15 μ in diameter, are found; at other times there will be numbers of large ones full of the characteristic inclusions and having diameters of 20–30 μ . However, with a little trouble all intermediate sizes may generally be found. At the ordinary room temperature movement is somewhat sluggish, consisting in the extrusion of lobose pseudopodia in various directions without any actual change of place. On warming, the amœbæ become very active and sometimes move rapidly across the field of the Microscope, and may extend themselves to 100 μ or more in length. The pseudopodia consist almost entirely of the refringent, slightly greenish ectosarc (figs. 9, 10, 12 and 13). There is no contractile vacuole, but the endosarc contains usually many food vacuoles. In small specimens the contained food is often bacteria, but sometimes one or two of the large inclusions, so typical of the larger amœbæ, may be distinguished. There has been much discussion as to the nature of these inclusions, and they have been described as such mysterious things as “something of Protozoan nature”—Craig (3)—or “bodies only present in inflamed tissue.” They are large, often 4–6 μ in diameter, refringent in the living, and stain readily with neutral red, Ehrlich’s hæmatoxylin, and methyl-green. That is to say, they do not behave as the red blood corpuscles ingested by *Entamoeba histolytica*, but seem to be composed of nuclear matter. In fact we consider that they are the nuclei of lymphocytes or other mononuclear leucocytes. In this connexion it is interesting to note that Mendel (10) has found 9 p.c. to 13 p.c. of the leucocytes in the gingival space to be mononuclears. On ingestion the small amount of cytoplasm would be rapidly digested, leaving the more indigestible nuclei in food vacuoles of the living amœba for a long time. Occasionally the branching nucleus of a polymorphonuclear leucocyte has been seen, also on one or two occasions a red blood corpuscle; but these are very rare. Though living amœbæ have been kept under observation for long periods (they will live in saliva or Ringer’s solution in vitro for eight or nine hours) we have not succeeded in seeing any large body actually ingested. An amœba has been seen to flow round a mononuclear leucocyte for a long time without finally accomplishing its ingestion. When feeding on bacteria amœbæ have been observed on several occasions to flatten themselves out and extrude numerous fine branching

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pseudopodia in all directions (fig. 10). These capture the small organisms, which may be seen passing towards the interior each in a tiny vacuole, and when the amœba reassumes its bulky form the endosarc appears to be a mass of small vacuoles.

The nucleus is seen with difficulty in the living (figs. 9 and 10), especially when much food is present. It shows up clearly, however, with such an intra-vitam stain as neutral red, and may then be seen to be of the vesicular form and to some extent compressible. There are generally a few granules of chromatin on its membrane. The small caryosome consists as a rule of a group of granules (figs. 12, 13); occasionally these form a ring (fig. 11), as is so often the case in *Entamœba histolytica*, which has a slightly larger nucleus. In *E. gingivalis* the nucleus is generally only 3 or 4 μ in diameter, but may be 5 μ or even 6 μ (fig. 11); possibly these latter are about to divide.

Reproduction, so far as we know, only takes place by binary fission, the nucleus dividing well in advance of the cytoplasm, so that two nuclei in a specimen have been seen on several occasions; these may be of different sizes, e.g. 3 and 4 μ respectively (fig. 13). Nuclear division is difficult to follow out in all its stages owing to the deeply staining inclusions. These appear to persist during the process, since two nuclei are found in amœbæ with vacuoles containing almost entirely digested food (fig. 13). If there be any sexual process or cyst production in the life-history of this amœba in the mouth it must be very rare. The only cysts that we have ever seen from the mouth have been obviously those of some free-living amœba of the *Limax* group (*Vahlkampfia*), i.e. small (8–12 μ in diameter), with a nucleus containing a large caryosome. The cysts described by Craig (3) from the mouth appear to be the same. Drs. Penfold and Drew, working in London, have recently cultivated an amœba from the mouth, but were disappointed to find that it had a contractile vacuole, and was obviously a free-living form and not *E. gingivalis*. That such cysts frequently find their way into the mouth from uncooked vegetables, etc., is proved by the fact that they so often reappear in the fæces.

All our attempts to grow *E. gingivalis* aerobically or anaerobically on various media and in association with different bacteria have been unsuccessful. (It may be of interest to point out that no parasitic amœba has ever yet been grown artificially.)

It is quite possible that there is more than one species of amœba in the mouth, but until some other stages in the life-history have been observed, inside or outside its host, it is premature to try to divide them.

It was in 1915 that Bass and Johns (1) produced an elaborate book in which they recorded the invariable presence of amœbæ in pyorrhœa lesions, and accused these Protozoa of being the specific

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cause of the disease owing to the following erroneous assumptions:—

1. That they burrow down into the tissues destroying the peridental layer and being found in greatest quantities in the deeper parts of the pockets. [It has already been mentioned that no signs of them can be found in the peridental layer, and they only go below the gum when carried there by the sheltering tartar.]

2. That they are not found in healthy gums. [This statement has been contradicted by their own country-woman, A. Williams, and her collaborators (14), who record that they found amœbæ in 29 p.c. of New York children with healthy gums. Lewald (9) in 1907 had even recorded them in a much higher percentage of what he called healthy mouths.]

3. That emetin, which has such extraordinary amœbicidal action on *E. histolytica*, causes the amœbæ to disappear from the gums, and is followed by an improvement in their condition. [This they admit themselves in a subsequent note (1, p. 143) not to be invariably true, and in none of the few cases that we have kept under observation during injection of emetin ($\frac{1}{2}$ –1 gr. a day for six days) have the amœbæ disappeared for any length of time. In fact not at all generally, and in one their disappearance for a few days we ascribe to the unaccustomed oral cleanliness, kept up under our supervision, as much as to the effect of the drug.]

Another argument against *E. gingivalis* being a potent cause of pyorrhœa is the fact that in advanced cases there are sometimes very few—many less than in other milder ones. In fine, the short notoriety, that the amœba had for about a year as the cause of pyorrhœa, has practically already died down.

Another Protozoan parasite sometimes present in pyorrhœa lesions is *Trichomonas*. This is presumably the same species, *T. hominis*, found in the human intestine, where, if in large numbers, it appears to give rise to a mild form of dysentery. In the mouth it is generally only present in small numbers, and we have not so far studied it specially. It lives, as would be expected, for a much longer time outside the mouth than the amœbæ. There is no evidence at all in support of its having any particular connexion with pyorrhœa.

DESCRIPTION OF THE LEPTOTHRIX OF THE MOUTH.

The white deposit to be found attached to and between the teeth is chiefly composed of colonies of Leptothrix, which we believe to be a pleomorphic organism. In examining the colonies great care should be taken to obtain them from the mouth in as unbroken a condition as possible. They have a high degree of tenacity, but if the material, scraped, say, from the tartar ridge, be teased out with needles or made into a smear as in making

ordinary bacteriological preparations, the broken parts of colonies will present a hopeless confusion. It is simply due to these methods of preparation that the fact has not hitherto been recognized that by far the greater proportion of the white deposit consists of colonies of this highly organized bacterium or parasitic alga. The easiest way to obtain unbroken colonies, or well-preserved portions of large colonies, is from extracted teeth. Part of the tartar ridge not disturbed by the forceps should be chosen, and some of the *Leptothrix* from the surface gently scraped off with a needle, and transferred to a drop of Locke's solution, or one of half Locke and half Gram's iodine, on a slide. If too thick a lump, it may be divided with a needle into smaller portions, and a cover-slip then gently pressed down to flatten out the preparation sufficiently to examine it. It must be remembered that the colonies have often been broken and disturbed whilst still in the mouth, unless they are in a sheltered position such as in a pyorrhœa pocket, from which beautiful little free colonies may sometimes be obtained. Balls of *Leptothrix* colonies pressed out from the tonsil-crypts also provide excellent unbroken material. To make permanent preparations of *Leptothrix*, portions of colonies should be carefully selected in Locke's solution or saliva under a dissecting microscope; they should then be arranged in a drop on one cover-slip and another pressed gently on top, and the whole immersed completely in some fixing solution, preferably freshly-made Carnoy or Schaudinn, and left there for about ten minutes, after which the cover-slips should be gently separated. One or other of them will probably show fairly good clumps fixed in a flattened condition. We have found Stephens' ink the best stain for colonies fixed in this way; and, for small separated branches, Giemsa or iron-hæmatoxylin (long method).

Leptothrix colonies vary immensely, but a fairly typical specimen of a small unattached well-developed colony (fig. 15) shows the following structure. The centre of the colony is a nodule of hard tartar, from which radiate bundles of long coarse threads divided into segments of variable length, which usually give a reddish-purple reaction with Gram's iodine (fig. 18). The ground-work between these bundles is filled in with granular or coccoid forms and traversed by numerous finer threads, most of which do not give the iodine reaction. We have never observed any true

EXPLANATION OF PLATE XV.

The magnifications stated for the following figures, Nos. 15-21, are approximate.

Figs. 15, 16, 17, 18, 20 and 21 represent optical sections. Figs. 17 and 20 show *Leptothrix* colonies flattened under cover-slips.

Fig. 15.—Diagram of a portion of *Leptothrix* colony, stained with iodine, which shows up the coarse bundles of threads. $\times 425$.

„ 16.—Diagram of a "bottle-brush" branch. $\times 2000$.

branching of the *Leptothrix* threads. The radiating bundles of relatively coarse threads divide and subdivide before reaching the periphery of the colony and may finally end in a few threads. These may project far beyond the general surface of the colony, and, being usually covered with coccoid forms, and finer and shorter threads set more or less at right angles to this axis, form a terminal branch, one of the many of very variable form with which the whole surface of the colony is thickly beset. The coccoid forms on the branches appear to be set in rows radiating from the axial threads. This arrangement is well seen in specimens of branches stained with Stephens' ink. In the solid mass or groundwork of the colony, however, their arrangement cannot be made out. Threads of *Leptothrix* often show terminal swellings or "clubs," both inside the colony and at the periphery. These are very prone to calcify (fig. 19).

Where a colony is growing on the surface of a tooth or other attachment it is of course modified in shape, but the bundles of coarse iodine-reacting threads always radiate from the centre of attachment, and there the hard tartar is deposited, chiefly by the progressive calcification of these threads, and is thus built up layer by layer as the colony grows and spreads. The forms of *Leptothrix* colonies vary considerably according to their position in the mouth. Those most branching and diverse are met with usually where the tartar ridge meets the surface of the gum-flap. Colonies situated above the surface of the gum margin, slightly higher up on the tooth, have usually less complicated branches. A variety frequently found on colonies in this position has been described as "flowering heads" by various writers (fig. 20). They are well illustrated in two microphotographs by Leon Williams (15), reproduced by Goadby (5). The young colonies found scattered over the surface of the tongue (fig. 17), and radiating from a core of a few dead epithelial cells, are often entirely composed of coccoid forms, these coccoid forms being set in regular rows, with alternating areas of finer or coarser granules, all apparently radiating from their centre of growth. The *Leptothrix* also flourishes on the surface of decaying dentine, its threads radiating from the decayed surface, and as a rule very little or no tartar is deposited when it is growing in this position. The *Leptothrix* also grows on foreign bodies in the mouth; thus we have found it growing abundantly on a fish-vertebra which had become lodged between two teeth in a cat. It has also been described by Leber and Rottenstein (8) growing on substituted human teeth, with decalcification taking place in exactly the same way as in ordinary caries. Under certain conditions as yet unascertained * the *Leptothrix* colonies develop

* We have not found this kind of branch on perfectly healthy extracted teeth or in healthy mouths. We always find it on extracted teeth from advanced cases of pyorrhœa. It usually abounds in pyorrhœa pockets.

branches of a peculiar type, as shown in our illustrations (figs 16, 21). To these we have given the name of "bottle-brushes." They consist of a few central threads with innumerable fusiform bodies, which are usually slightly curved, so set upon this stem as to make it resemble a little fir-branch. Sometimes these fusiform bodies appear to be firmly attached to the branch, but in other specimens they may be watched coming off the axial threads in clouds, and in this condition they appear to be motile. They have often already divided into a diplobacillary form, while still attached to the stem. Branches are frequently found which have already shed these bodies from their distal portions, but are still closely beset with them lower down. In this condition the distal portion of the branch is seen to be covered with little coccoid bodies which at a later stage appear to have budded off more coccus forms in the typical radiating rows, while the lower portion may still be covered with fusiform bodies. This fusiform bacillus appears to be the one associated with a spirochæt in Vincent's angina.* With regard to the "iodine reaction" recommended by so many authors for differentiating the different species of bacteria found in the mouth, we have made the following observations:—The radiating bundles of coarse threads usually give this purple-red reaction; the intensity, however, varies very much, from nearly black to a hardly discernible tint. If long coarse threads project singly far beyond the surface of the colony they almost invariably do not give the reaction. Some of the finer threads in lobose branches of a colony usually give it, but as a rule it is only a few at intervals that do so. Sometimes a whole colony fails to give the reaction. Occasionally whole branches stain deeply with iodine—central threads, shorter radiating threads, coccus-form, and all. In a cat we have even found bottle-brushes with the attached fusiform bacilli staining right through. Often a few areas of the coccus-form in a colony give the reaction. It seems to us that the iodine reaction may be regarded as merely showing the presence of a food substance which may be stored in different parts of a colony.

* On a few occasions it more resembles the *Bacillus necrosis* (see Ellermann (4)), that is to say, it is longer, and has a striated appearance due to the distribution of the granular contents.

EXPLANATION OF PLATE XVI.

- Fig. 17.—Young colony of *Leptothrix* from the tongue, growing on a core of dead epithelial cells, which are already partly calcified. Drawn from a fresh preparation stained with iodine. The coarse threads leading to little branches gave the iodine reaction. $\times 300$.
- „ 18.—Two coarse threads of *Leptothrix*, stained with iodine, showing the granules giving the purple reaction in the segments. $\times 3500$.
- „ 19.—A group of partly calcified clubs, from the periphery of a *Leptothrix* colony. $\times 625$.

The cause of the deposit of hard tartar was described by W. D. Miller (11) as a chemical reaction. The CO_2 in the saliva was said to escape on reaching the mouth, and, the alkalinity of the saliva thereby becoming greater, the salts, being then less soluble, were deposited. As proof of this, test-tube experiments were adduced, and the fact that more tartar is deposited on those teeth near the openings of the salivary ducts into the mouth was supposed to settle the matter. Exactly the same explanation is given in "The Science and Practice of Dental Surgery," by Bennett, in 1914. In this work it is admitted, however, that the deposit of hard tartar in some positions, notably on the roots of teeth which had apparently no lesion leading to the mouth-cavity, presented difficulties. We have already described the positions in which hard tartar is found, but we have never found any unless the *Leptothrix* was there depositing it. The abnormal amount of tartar formed in pyorrhœa is a marked feature of the disease, both in man, cat, dog, and also, we hear from Dr. Broom, in the acute pyorrhœa of the golden moles of South Africa, *Chrysochloris hottentota* and *asiatica*, where not only the whole of the milk-dentition is rapidly lost from this disease, but the germs of the permanent teeth are so injured that they fail to erupt. In the tonsil-crypts we have found the *Leptothrix* growing in large colonies, some of them depositing hard tartar in their centres. It is probable that tonsil-stones are due to this cause. It will possibly be found on further investigation that some calcareous stones from other parts of the body have the same origin. Those occurring in the salivary ducts are particularly likely to be of this nature.

It is worthy of note that the sore surface in pyorrhœa is always found opposite the tartar ridge, and consequently in juxtaposition to the colonies of *Leptothrix* with which the ridge is covered. The *Leptothrix* follows down the recession and abounds in the pockets formed. According to R. von Jaksch (7) it grows abundantly in the lungs, in fetid bronchitis and gangrenous lung affections. Von Jaksch says that by *Leptothrix* he means the *Bacillus maximus buccalis* of Miller. If we have correctly identified the fusiform body, it appears to have some relation to Vincent's angina and hospital gangrene. We merely indicate these positions in which the *Leptothrix* is found in order that it may, we hope, not be entirely overlooked in future as a possible cause of other diseases as well as of pyorrhœa.

The *Leptothrix* was described by many early investigators with varying degrees of accuracy. The first to study it with detailed attention was Vicentini (13). His descriptions of what he called *Leptothrix racemosa* (including under this name both species of *Leptothrix* described by Miller as well as other organisms) were not altogether correct, and its pleomorphism unfortunately led him to the remarkable conclusion that all forms

of human pathogenic bacteria were derived from this one parent plant. It is to his preposterous claims for the organism that we must attribute the neglect of later investigators to follow up his observations. Leon Williams (15), one of his contemporaries, dealt with a few points in Vicentini's work, and described a method of staining the colonies with methyl-violet. W. D. Miller, writing about the same time, would not allow the existence of the organism at all as such, but divided it up into several kinds of bacteria. His classification and nomenclature have been partially adopted by later writers on the subject. We only propose here to refer to one of his kinds—namely, *Bacillus maximus buccalis*—in the hope of clearing up some of the confusion which has arisen over the use of the name. Miller applied the term to the coarse iodine-reacting bundles of threads which we have just described in the *Leptothrix* colonies. His description and illustrations leave no doubt upon this point. He says it most frequently occurs as parallel-running or crossing bundles of segmented filaments which give the iodine reaction. Goadby (5), while accepting Miller's classification of the so-called "ungrowable" mouth bacteria, describes under *B. maximus* (in which he includes *B. maximus buccalis* and *Leptothrix buccalis maxima* of Miller) an organism which he has grown on various media. His description, however, in no wise tallies with Miller's. Goadby's organism is motile and forms endogenous spores; a few of its threads give the granulose reaction on certain media; he says nothing about its running in parallel bundles. Bennett (2) describes *B. maximus buccalis* as a large bacillus with segments 5–6 μ long (Miller gave them as 2–10 μ) forming definite chains, which sporulate, but only when grown outside the mouth! He says it is non-motile, and that he means the organism described by Miller as *B. maximus buccalis*. He also says nothing about its iodine reaction nor about its running in parallel bundles. We wish to suggest that the *B. maximus buccalis* of Miller has not yet been grown artificially, and that it cannot be so grown until the whole *Leptothrix* colonies, of which it forms an integral part, have been cultivated on artificial media.

There seems to be no doubt that the fusiform bacillus has been grown in pure culture, and also that it sometimes grows out into threads. By supplying the cultures with various substances

EXPLANATION OF PLATE XVII.

- Fig. 20.—Part of a colony of *Leptothrix*, with "flowering head" branches. Drawn from a fresh preparation. The axis of this kind of branch consists of a single swollen thread. Some branches are seen in optical section. $\times 625$.
- „ 21.—A "bottle-brush" branch. Drawn from a preparation stained with Stephens' ink. $\times 600$.

possibly necessary for its growth, we intend to attempt the cultivation of complete colonies of *Leptothrix*; this we recognize to be the only conclusive proof that the fusiform, coccoid, and filamentous forms are all parts of the same organism.

We may sum up by stating that pyorrhœa lesions differ from other suppurating sores in that no organisms appear to invade the tissues. Possibly they are kept out by the normal flow of leucocytes being so greatly increased as soon as the epithelium is injured. In any case we cannot yet definitely accuse any organism of being the primary cause of the gingivitis with which we feel sure the disease begins, but are inclined to incriminate the *Leptothrix*.

In conclusion, we should like to express our great indebtedness to the authorities of the Radcliffe Infirmary, Oxford, especially Major Gibson, D.M., for the use of a laboratory in that institution; also to the dental surgeons who hold clinics at the Infirmary and have provided us with much material. Mr. Kendrew and Mr. Pettey have especially given us assistance. Mr. E. S. Goodrich has given us much valuable advice and help during the progress of the work; and we are also much indebted to Sir William Osler, not only for suggesting the study of this disease, but for the great help he has given us throughout the course of the research.

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