AN INVESTIGATION INTO THE ÄETIOLOGY OF DENTAL CARIES. I: THE NATURE OF THE DESTRUCTIVE AGENT AND THE PRODUCTION OF ARTIFICIAL CARIES.

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CONSIDERING the wide-spread and almost universal distribution of dental caries, it is not a little surprising that we have no definite knowledge of the causal agent or agents. Numerous theories have been propounded in explanation, details of which are to be found in the various monographs which have appeared from time to time. It is therefore not proposed to deal with these views individually here. Perhaps the parasitic theory, or, as it is more aptly expressed by Miller (1890), the chemo-parasitic theory, has received most support.

The chemo-parasitic theory of dental caries may be regarded as having originated with Miller's brilliant researches. The purely inflammatory view is of course much older. Miller regarded acids produced by the fermentative action of bacteria as the primary factor in dental decay. He, however, lived in advance of his time; his bacteriological technique was exact, but bacteriological knowledge was not sufficiently advanced to profit by his researches or to identify the organisms he found in the lesions. Many of the bacteria observed by him failed to grow on the media at his disposal, so that much of his study was purely morphological. He definitely showed that mouth bacteria, by their action on carbohydrates, could produce sufficient acid to injure teeth. Since Miller's time most of the work seems to have consisted in enumerating the various bacteria found in the mouth and in dental caries—researches which might be more aptly described as investigations into the bacterial associations of dental caries. Miller enumerated a very large number of bacteria and classified them according as to whether he was able to grow them or not. Goadby (1903) in his valuable monograph described some eighteen types, which he subdivided into acid-producing types and dentine liquefiers. Kligler (1915) identified ten different groups. Howe and Hatch (1917), although no exact details or actual figures are given, consider that two types of bacteria, which they group together under the name "Moro-Tissier," are most commonly associated with dental caries.
DENTAL CARIES.

NATURE OF THE DESTRUCTIVE AGENT.

Decalcification.

In taking up this research on dental caries we were of opinion that if bacteria played an important rôle, then it was necessary to find micro-organisms which were capable of softening enamel, which process histopathological research has shown to be the initial lesion in caries. For the present, of course, we do not intend to deal with any of the predisposing causes, although the part played by structural deformities, whatever be their origin, cannot be denied. We have already noted that Miller considered that the production of acid by bacteria was the initial process. We therefore have endeavoured to discover a bacterium or group of bacteria present in dental caries which can produce sufficient acid to decalcify enamel and dentine. Once decalcification has been produced, the path is left open for almost any micro-organism to invade the dentine.

It is reasonable to suppose that the bacteria which produce large quantities of acid will be able to live in relatively high concentrations of acid. Therefore, bacteria which are able to decalcify enamel must be able to live in a very acid medium. This is briefly the thesis on which we have based this research and the basis of our technique to discover the bacterium.

The first procedure was to determine what degree of acidity is required to decalcify tooth enamel. This can be arrived at in two ways—experimentally and theoretically.

Experimentally the simplest procedure is to place normal teeth (i.e. teeth showing no caries) into solutions of different degrees of acidity. In our experiment we used acid nutrient broths of different pH values. The acid broths varied from pH 5 to pH 1, and the teeth were left in for thirty-four weeks. The teeth had previously been sterilised in the broth by autoclaving. At the end of the period the degree of whitening or opacity of the enamel was noted. It was found that no change was to be seen in those teeth placed in broths of pH values higher than 4, and only such a minute trace as to be negligible in pH 4 (Fig. 1).

The theoretical determination is much more difficult, as the formulae have all been constructed to deal with elements in solution. Dealing with the carbonate radicle, it is possible to arrive at an approximate value by the calculation of the pH value of a saturated solution of carbonic anhydride. A saturated solution such as might occur in the mouth would give a solution corresponding to about N/1000.

From the formula $\sqrt[4]{K_1 \times 1}$, where $K_1$ = the dissociation constant, we get a pH value of 4.8 for such a solution.

The chemical affinity of acids in equivalent solutions is proportional to the dissociation constants, therefore we can assume that the acidic values must be in excess of this pH value before the carbonic oxide can be displaced from a compound, as it is reasonable to suppose that teeth are not affected by the CO$_2$ of the saliva.

Technique of Isolation.

Carious teeth were obtained from the Dental Out-Patient Department and from two private practices in order to include as many different types of
mouth conditions as possible. A tooth was held in sterilised forceps and passed through the flame a few times; with a sterile scalpel the superficial part of carious dentine was removed and the deeper part emulsified in broth pH 7·6. After twenty-four hours' incubation broths of varying degrees of acidity were heavily inoculated, and from these agar plates were inoculated after twenty-four or forty-eight hours' incubation. At first the acid broths used varied from pH 7 to pH 4·5, but as a large variety of organisms grew in each tube higher concentrations of acid up to pH 1 were tried. Bacilli were isolated with some frequency from pH 3 broths, and on one occasion from pH 1, but on the whole we found there was no need to employ such degrees of acidity. Later the carious dentine was emulsified in pH 6 broth direct, and after twenty-four hours' incubation the more acid broths of pH 4·5 to pH 3 were inoculated. The most successful method, however, was to emulsify the carious material in pH 3·5 broth in the first case, as in this the vast majority of tooth organisms fail to grow.

Nature of Micro-organisms Isolated.

The organisms isolated by the above method fall, morphologically, into two main groups: (a) Type I, a long thin bacillus, 0'75 x 2–3μ, which occurs singly, in pairs or chains, having a marked tendency to parallelism or palisade formation in dried films (Figs. 2 and 3); and (b) Type II, a shorter bacillus, 0'75 x 1–2μ, usually occurring in chains (Fig. 4). After prolonged subculture on agar Type I fails to form chains. Both organisms are non-motile and Gram-positive; both are aerobic and facultative anaerobes (in gelatin stab cultures there is equal growth at the top and the bottom). In broth there is in most cases uniform turbidity, but in a few cases the growth settles to the bottom. On ordinary agar the colonies may appear as minute points after twenty-four hours' incubation, but often do not become visible till after forty-eight hours; they are small, round, greyish and opaque, with a finely granular appearance under the low power and a regular outline (Fig. 5). In size the colonies are about 0·5–1·0 mm. in diameter; on serum agar rather larger, up to 2·0 mm. In a gelatin-agar shake culture the colonies have a rough biconvex appearance which might be described as being "tam-o'-shanter" shaped (Fig. 6).

When first isolated subculturing should be carried out once a fortnight, as the organisms have a tendency to die out, but after prolonged subculture once a month is sufficient.

Type I was isolated in pure culture from the deeper layers of carious dentine from 38 out of 50 teeth examined, and Type II from 18 teeth out of 50. In some cases the growth of mouth organisms in the original broth culture outgrew the above two types to such an extent that they were not isolated although morphologically similar organisms were seen in films; inoculation into acid broth direct obviates this difficulty. Including these cases, Type I "occurred" in 44 cases, i.e. 88 per cent., and Type II in 21 cases, i.e. 42 per cent. In some cases only one type was isolated from each tooth, in others both types; one or other or both types were isolated in pure culture from 88 per cent. of cases and "occurred" in 96 per cent.

As regards the fermentation of sugars, of the 38 strains of Type I isolated 33 formed acid and no gas in glucose and lactose, no acid nor gas in saccharose
and acid and clot in milk. The milk usually became acid on the second day and was firmly clotted on the third, with all except the top quarter to half an inch completely decolourised. Four of the remaining five strains formed acid in saccharose and glucose, but differed in their reactions in lactose and milk; one strain formed acid in glucose alone. The action of Type II on sugars is nothing like so constant; seven out of the eighteen strains isolated formed acid and no gas in glucose, lactose and saccharose, and acid and clot in milk, the effect on the last-named being similar to that of Type I. Three strains formed no clot in milk after prolonged incubation, and two strains had no effect at all on milk. The remaining six strains varied considerably in their reactions (Table I).

Table I.—Sugar Reactions.

<table>
<thead>
<tr>
<th>Type</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Saccharose</th>
<th>Milk</th>
<th>Gelatin</th>
<th>Indol</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>Ac. Cl.</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>0</td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>0</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Type II</td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac. Cl.</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>Ac.</td>
<td>0</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ac.</td>
<td>0</td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td></td>
<td>Ac.</td>
<td>0</td>
<td>0</td>
<td>Ac. Cl.</td>
<td>0</td>
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<td></td>
<td>Ac.</td>
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<td></td>
<td>Ac.</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

In no case was gelatin liquefied by either type, nor was indol formed; twenty-six strains were also inoculated into dextrin and dulcite without the formation of acid or gas.

We propose to give the name B. acidophilus odontolyticus I and II to these bacilli.

Occasionally other organisms were isolated, but with no regularity.

Relation of the Two Types to other Bacteria found in Dental Caries.

Morphologically Type I very closely resembles one of the organisms illustrated by Miller in an excellent micro-photograph of artificial caries. Howe and Hatch state that the predominating group of organisms in dental caries is the Moro-Tissier group. B. acidophilus (Moro) as depicted by them bears morphological resemblance to Type I. The sugar reactions, however, do not agree, since he states that most strains ferment saccharose; the action on milk is not mentioned, but Moro (1905) states that cow's milk is coagulated while human milk is not.

Kligler, working on oral micro-organisms, isolated fifty-eight strains of B. acidophilus (Moro), which he states ferments glucose readily with the formation of a considerable quantity of acid, lactose not so readily and
saccharose not constantly. As regards milk, the lower part is coagulated first and litmus milk becomes decolourised. Summarised, the following are the reactions of the strains isolated by him:

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Saccharose</th>
<th>Milk</th>
<th>Gelatin</th>
<th>Indol</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>A.C.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>+</td>
<td>A.C.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

That is, 60 per cent. agree in the sugar reactions with our Type I.

The Occurrences of Types I and II in "Normal" Mouths.

In order to form some idea as to the frequency of occurrence of decalcifying organisms in the mouths of persons who had at one time or another suffered from caries, ten persons thoroughly rinsed their mouths with 10 c.c. of normal saline, and the fluid was collected and deposited into an equal quantity of double-strength broth. From these cultures Type I organisms were isolated in 30 per cent. of cases and Type II in 20 per cent. This experiment will be further described in a later paper, along with the exact relation of the bacilli and their distribution.

Agglutination.

To test agglutination properties, two strains of Type I were inoculated repeatedly into rabbits; each strain agglutinated with its own antiserum up to a dilution of more than 1 in 2560. Sixteen strains were put up against serum A, and 14 against serum B, with the following results:

<table>
<thead>
<tr>
<th>No. put up.</th>
<th>1/40</th>
<th>1/80</th>
<th>1/160</th>
<th>1/320</th>
<th>1/640</th>
<th>1/1280</th>
<th>1/2560</th>
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</thead>
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<tr>
<td>Serum A</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Serum B</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

These results include strains of both types, but in each case the organisms which were agglutinated in the higher dilutions belonged to Type I.

* Final pH Values of Cultures.

It has been stated above that we found that a concentration of acid greater than pH 4 was necessary to effect decalcification of enamel, therefore it was essential to discover whether the organisms isolated were capable of forming this amount of acid by the fermentation of carbohydrates. Nine strains picked at random were inoculated into glucose broth of pH 7.6 and placed in the incubator until they died out, and the final pH values were estimated on the hydrogen electrode; these varied between pH 3.4 and pH 2.2, the average being pH 2.75. It thus appears that organisms of either Type I or Type II are able to elaborate enough acid from the fermentation of carbohydrates to attack the enamel of teeth. A seven days culture of B. coli in glucose broth gave a final pH value of 4.0.
DENTAL CARIES.

PRODUCTION OF ARTIFICIAL CARIES.

Decalcification of Enamel.

Attempts were next made to produce caries artificially by the action of the organisms isolated. Non-curious teeth were placed in broth and 2 per cent. glucose broth in 3" × 1" tubes and sterilised; various strains were then inoculated into the broth, S. salivarius in both ordinary broth and glucose broth and B. coli in glucose broth being used as controls. Every eighth day the teeth were removed and placed into fresh uninoculated broth under sterile precautions, so as to approximate somewhat to the conditions in the mouth and prevent the teeth remaining continually in an acid medium. As pointed out by Miller, the first sign of caries is the loss of transparency of the enamel, which becomes opaque; this he attributes to pure decalcification. This opacity is clearly seen in the tooth in Fig. 7, which had been in a broth culture of Type I for seven weeks. Figs. 8 and 9 show low- and high-power magnifications of a longitudinal section from a tooth, the upper half of which had been in a glucose broth culture of Type I for seventeen and a half weeks. The organisms have penetrated the dentinal tubules for a considerable distance from the pulp cavity side, which fact proves that the appearance is artificial, since "natural" caries never starts from the pulp cavity and works outwards. In the plates not only can the organisms be seen passing down the tubules, but also a large liquefaction focus can be seen. Fig. 10 is another tooth in which the dentinal tubules have been cut transversely; some of these are seen to be packed with organisms and dilated to a far greater diameter than the empty tubules, which have simply undergone decalcification during the process of embedding. This tooth shows the result of eleven weeks in a glucose broth culture. In all cases observed up to the present the first sign of caries—that is, the opacity of the enamel—occurs on the cutting edge of incisors or canines and on the tip of the cusps of molars and premolars; this fact was also noted by S. P. Mummery (1909–10) when working with teeth suspended in 0.075 per cent. lactic acid. The control teeth in cultures of S. salivarius show no macroscopical changes after five months, nor does the control in a culture of B. coli after two and a half months; these experiments are being allowed to continue yet further. All the teeth in broth cultures show some slight opacity of the enamel. Further experiments are also being carried out in glucose broth with the lower part of the tooth sealed over in order to produce caries through the enamel alone. Commencing caries of the dentine from the enamel side is indicated in Figs. 12 and 17. Fig. 11 represents a section of a tooth with the tubules cut transversely, showing the effect of placing decalcified non-curious dentine in a broth culture of S. aureus for three weeks. No organisms can be seen but the proteolytic enzyme has had a very marked effect, producing enormous cavities in the dentine, far larger than the liquefaction foci which appear in sections of "natural" caries. For the sections and micro-photographs in Figs. 12 to 19 we are indebted to Mr. J. Howard Mummery; these teeth had been in glucose broth cultures for fourteen weeks.
Penetration and Liquefaction of Decalcified Dentine.

Although in no case was gelatin liquefied nor indol formed by any of the organisms isolated, Figs. 8, 9 and 16 clearly show the liquefaction foci as described by Miller. After completely blocking the tubules the organisms have produced large cavities in the decalcified dentine in several places. It thus appears that when completely deprived of carbohydrates the organisms isolated can exert a liquefying action on the collagen matrix of the dentine, although as yet we have been unable to obtain any action on gelatin.

Animal experiments are being carried out, the results of which will be discussed in a later paper.

DISCUSSION OF RESULTS.

In order to facilitate the correlation of our results with the findings in natural caries a short account of the natural process may not be out of place. The main histological features of caries are definite in type and well known.

Dental decay is, with a few exceptions, universally present in both primary and secondary dentition. The condition commences in the crowns of the teeth usually at the points of contact or in the fissures. Caries of the enamel appears as a white opaque area; this is soon followed by fracture of the enamel prisms, disintegration of the enamel and exposure of the dentine. The dentine is then involved, decalcified, softened and destroyed. Microscopically the bacteria can be seen to have penetrated the dentinal tubules, which are enlarged and breaking down with intercommunicating areas. Ground sections of carious teeth at different stages of the disease show the whole process very clearly. There are, however, variations according to the structure of the teeth.

In many of these preparations bacilli morphologically resembling those we have described are seen alone or with other bacteria. Some of Miller's original photographs show this very clearly.

Enamel changes in artificial caries.—The primary lesion produced by prolonged contact with cultures of our micro-organisms is a whitish opacity similar to that seen in natural caries. Sections show that this change is not uniform in that in certain areas the penetration is much more marked than in others; this is clearly seen in Figs. 12 and 17—sections of a tooth which had been in a broth culture for seven weeks. The sections also show slight undermining of the enamel. Under the microscope the enamel prisms are granular and in places fragmented.

Dentine changes in artificial caries.—Miller noted that the first change was an area of decalcification in advance of the organisms. Fig. 17, a ground section, shows staining of the decalcified dentine, whilst the infected dentine is still more deeply stained. So far we have not been able to get a good specimen showing this passage from the enamel side, but this is mainly due to the fact that we did not leave the teeth long enough in contact with the cultures.

The characteristic mode of spread, however, is well shown in Figs. 8 and 9, and only differs from true caries in that the process started from the pulp surface. Microscopically the bacilli are found to be spreading down the dentinal tubules in a manner identical with the natural process. There is
Teeth placed in acid broths of pH 5·0 to pH 1·0 to show the acidity at which enamel is attacked. Those in pH 1, 2, 3, enamel attacked; those in pH 4, 4·5, 5, enamel not attacked.

Type I bacillus; smear preparation from agar slope culture, showing palisade and chain formation. Gram. X 1000.

Same description as Fig. 2.

Type II bacillus; smear preparation from agar slope culture. Gram. X 1000.

McIntosh, James and Barlow. Dental Caries.
**Fig. 5.**
Surface colonies of Type I on agar, showing finely granular appearance. × 18 and × 45.

**Fig. 6.**
Colonies of Type I in gelatin-agar shake culture; "tam-o'-shanter" colonies. × 45.

**Fig. 7.**
Artificial caries, showing opacity of enamel. Tooth in broth culture of Type I bacillus for 7 weeks.

**Fig. 11.**
Transverse section of dentinal tubules to show the effect of liquefaction of decalcified dentine by *Staphylococcus aureus*. (Zeiss obj. ½ in., oc 4.)

McIntosh, James and Barlow. Dental Caries.
Fig. 8.
Artificial caries; organisms in the dentinal tubules and liquefaction focus. Longitudinal section.
(Zeiss obj. 1/4 in., oc. 4.)

Fig. 9.
Same specimen as Fig. 8. (Zeiss obj. 1/4 in., oc. 4.)

McIntosh, James and Barlow. Dental Caries.
Artificial caries; ground section, showing caries of enamel and organisms passing along the amelo-dentinal junction. \( \times 30 \).

Section of artificial caries; individual bacilli in the dentinal tubules, also liquefaction focus. \( \times 700 \).

Artificial caries; section showing spread of bacilli along the dentinal tubules, which are considerably dilated. \( \times 40 \).

Same description as Fig. 13. \( \times 1000 \).

Section of artificial caries; large liquefaction foci. \( \times 400 \).

McIntosh, James and Barlow. Dental Caries.
Fig. 19.

Artificial caries: organisms in the tubules and large liquefaction foci. Polychrome methylene blue. × 400.

Fig. 17.

Ground section showing caries of the enamel and the undermining of the enamel by the organisms, and commencing destruction of the dentine. × 10.

Fig. 18.

Artificial caries starting from the pulp cavity: tubules packed with organisms and showing liquefaction foci. Polychrome methylene blue. × 40.

McIntosh, James and Barlow. Dental Caries.
also a true widening of the tubules with destruction and formation of liquefaction foci. Those foci stain differently with polychrome methylene-blue from the tubules merely blocked with organisms, the former being greenish in colour and the latter blue (Fig. 19).

The production of liquefaction foci strongly supports the aetiological importance of the bacilli we have described. In addition to producing sufficient acid to decalcify teeth and to initiate dental caries they can liquefy dentine. In natural caries, however, it is probable that this liquefaction process may be aided by a secondary infection of the dentine by proteolytic bacteria, although actively proteolytic bacteria such as the anaerobe B. sporogenes were never found. Placing decalcified dentine in cultures of proteolytic bacteria produced an extreme degree of proteolysis with the tubules all widely dilated (Fig. 11), and if the action be prolonged the dentine is completely dissolved. Such a condition is never seen in natural caries.

In a further paper we propose to deal with some of the secondary factors in the production of dental caries.

CONCLUSIONS.

(1) The examination of selected carious material showed the constant presence of a definite type of bacillus.

(2) The bacilli are capable of producing a high degree of acidity by the fermentation of carbohydrates. The average final pH value of nine strains was 2.75, which is sufficient to decalcify teeth.

(3) Teeth left in contact with pure cultures over prolonged periods showed changes almost identical with those found in natural caries.

(4) Such teeth show erosion of the enamel with penetration of the dentinal tubules and the formation of liquefaction foci.

(5) The bacilli, to which we propose to give the name B. acidophilus odontolyticus, in their resistance to and formation of acid resemble the “acidophilus” group of Moro; biologically, however, there are several points of difference.

Finally we wish to express our indebtedness to Mr. J. Howard Mummery for the great trouble he has taken in the preparation of a number of the sections and drawings, and to Mr. J. Q. Rowett, who has generously defrayed the expenses of this research.

REFERENCES.

GOADBY, K.—(1903) ‘The Mycology of the Mouth,’ London: (Longmans, Green & Co.).

HOPE, F., AND HATCH, RUTH.—(1917) Dental Cosmos, 59, 961.


