ON THE BACTERIAL FACTOR IN THE ÆTIOLOGY OF DENTAL CARIES.

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ALTHOUGH many factors may enter into the ætiology of dental caries, there is little doubt that the lesions are the result of the action of bacteria. The initial process is one of decalcification, and Miller's theory—that this decalcification is produced by acid elaborated by the bacterial fermentation of carbohydrates in the mouth—is now almost universally accepted.

The object of the present investigation, which was undertaken at the instance of the Dental Diseases Committee of the Medical Research Council, was to ascertain if there was regularly present in early carious lesions a specific organism or group of organisms which could elaborate acid in sufficient quantity for the initiation of the process under conditions not very different from those obtaining in the mouth.

Goadby (1903) came to the conclusion that "dental caries is not a specific disease due to a specific micro-organism," but in more recent times Howe and Hatch (1917), and also McIntosh, James and Lazarus-Barlow (1922), have drawn attention to the fact that *Bacillus acidophilus* is constantly present in the carious lesions. McIntosh, James and Lazarus-Barlow produced lesions resembling those of caries in healthy teeth kept in glucose broth cultures of this bacillus, and this is the only direct evidence that has been produced up to the present to establish an ætiological relationship between a specific group of organisms and dental caries.

PRELIMINARY INVESTIGATIONS.

An attempt to determine the part played by *B. acidophilus*† in the ætiology of dental caries was first made. The teeth examined by Howe and Hatch, and McIntosh, James and Lazarus-Barlow were in a comparatively advanced stage of caries, with cavity formation, and the conditions in such cases are very different from those which obtain at the initiation of the disease.

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† There is, apparently, a group of acidophile bacilli, the members of which, while differing from one another in minor points of morphology, in fermentation reactions and serologically, all have the general characteristics of the *B. acidophilus* of Moro. No attempt was made to analyse the group, and the name "*Bacillus acidophilus*" is used to designate any non-sporing, Gram-positive bacillus which produces acid in glucose broth and will grow on glucose agar with a reaction of pH 4.

The cavities, open to the mouth, retain considerable quantities of food material which rapidly ferments and becomes acid, and the resulting acidity encourages the growth of *B. acidophilus* while inhibiting that of other bacteria. The question to be decided was, therefore, whether *B. acidophilus*, which is a constant inhabitant of the mouth, is the cause of the carious lesions or merely a secondary invader of an already established process.

Technique of isolation.—Four teeth were in an advanced stage of caries, and in their case the technique of McIntosh, James and Lazarus-Barlow was adopted. The others were dipped momentarily into boiling water, in order to sterilize the surface as far as possible, wrapped in a piece of sterile sheet lead, placed in a vice and sawn with a sterile hack-saw, in most cases through a healthy portion, until it was thought that the base of the carious area had been reached, when they were split by inserting a sterile chisel into the saw-cut. The deepest part of the carious material was then scraped out with a sterile excavator and inoculated into glucose broths at pH 4 and pH 5·6.

Results.—Twenty-four teeth were examined, the majority being in an early stage of decay. *B. acidophilus* was isolated from 8, 4 of which were cases of advanced caries, while the other 4 had very shallow foci in the dentine with considerable erosion of the enamel, and it was impossible to obtain the material for culture without encroaching on the surface.

The above results indicated that *B. acidophilus* was not regularly present in the earlier lesions of caries. A second series of 24 teeth was, therefore, examined in order to confirm this, and at the same time to ascertain what organisms other than *B. acidophilus* could be isolated from such lesions.

The technique adopted was similar to that used in the previous experiments, but the carious material, instead of being inoculated into acid glucose broth, was plated on glucose agar at pH 5·6 and pH 7.

B. acidophilus was isolated from 7 teeth, three being in an advanced stage of caries, and the other 4 having shallow foci with considerable erosion of the enamel. The results of the previous experiments were, therefore, confirmed.

On the pH 7 plates an organism growing in peculiar dull, greyish-white colonies attracted attention. It was isolated with considerable frequency, especially from early cases, and occurred once in pure culture. On investigation it proved to be a streptococcus of very distinctive characteristics, and although not acidophile, an extremely active producer of acid. It has not, apparently, been previously described, and the name *Streptococcus mutans* is proposed for it.

The only organisms other than *B. acidophilus* and *S. mutans* isolated with any degree of frequency were the ordinary mouth streptococci, mainly of the *S. salivarius* type.

INCIDENCE OF *STREPTOCOCCUS MUTANS* IN CARIES.

As *S. mutans* appeared to be fairly constantly present in early carious lesions, and as it was found to be a powerful acid producer, 50 teeth in the earlier stages of decay were examined in order to ascertain the relative

frequency with which it could be isolated; 40 of these were cases of fissure caries, selected because there is frequently present in this variety of the disease a considerable area of decay in the dentine with little or no loss of enamel substance, and material more or less free from secondary infection and from contamination with surface bacteria could be obtained. In the other 10 the carious foci were on an approximal surface, being confined to the enamel in nine.

Technique of isolation.—In the fissure cases a modification of that previously used was adopted. The roots were cut off and the crown sawn from below; the carious material was drilled out with a fine drill, suspended in a drop of normal saline, and plated on glucose agar at pH 7. Figs. 1 and 2 are photographs of decalcified sections of two teeth, showing the invasion of the dentine by bacteria and the position from which the material for culture was taken; in a number of cases, however, it was not possible to obtain the material so deeply, as the lesions did not extend far enough into the dentine. Two of the approximal cases were treated in a similar manner, but the remainder were simply drilled from the outside. Plates were examined at the end of forty-eight hours, and, after colonies had been picked off, a general scraping was inoculated on glucose agar at pH 4 in order to isolate *B. acidophilus* if present.

Results.—*S. mutans* was isolated from 36 of the 50 teeth. Of the 40 fissure cases it was isolated from 27, being found in pure culture in 8; 4 plates were sterile, and on practically all the remainder colonies identical with those of the streptococcus were seen, although it could not be isolated. It is rather difficult at times to pick these colonies off the surface of the medium without their becoming contaminated from neighbouring ones, and almost impossible to separate *S. mutans* from a contamination, on account of the slowness of its growth and the rapidity with which it becomes overgrown. In 7 of the cases from which pure cultures were isolated the enamel was apparently intact and the material for cultivation obtained fairly deeply in the dentine, while in the eighth, although there was some erosion of the enamel, the material was obtained very deeply and at the extreme edge of the lesion. *S. mutans* was isolated from 9 of the approximal cases, and numerous colonies were seen on the tenth plate. Although, as might be expected, a large variety of organisms grew on these plates, *S. mutans* very markedly predominated in the great majority of cases, sometimes outnumbering all others in the ratio of 4 or 5 to 1.

B. acidophilus was present in 9 fissure and 5 approximal cases, all the former having foci of caries so shallow that the enamel, which was in most cases eroded, broke away with the drill and the surface of the tooth was encroached on in taking the specimen. In 11 of the cases from which *B. acidophilus* was isolated, *S. mutans* was also present.

Streptococci of various kinds, and more rarely staphylococci, were the only other organisms which appeared on the plates with any degree of regularity. There is little doubt that they were contaminations from the surface, as in cases from which the specimens were obtained deeply they were present in extremely small numbers, if at all. It is obviously not easy to sterilize the surface of a tooth thoroughly without allowing the heat to penetrate deeply enough to kill the bacteria in the carious focus.

STREPTOCOCCUS MUTANS.

Morphology.—When grown on a medium with a neutral or alkaline reaction, *S. mutans* is a non-capsulated coccus, about 0·75 μ in diameter, growing

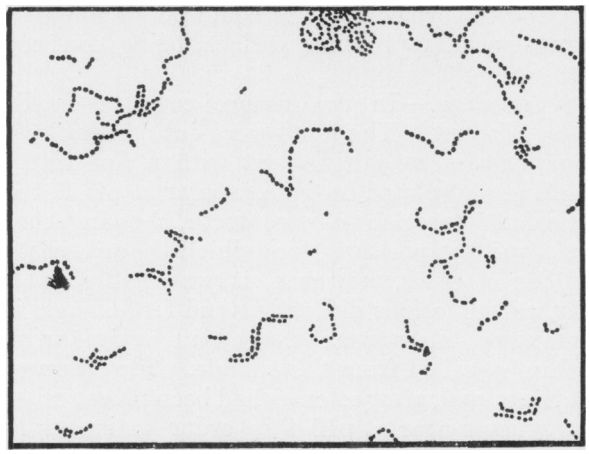

FIG. 3.—*S. mutans.* 24-hour broth culture. × 1200.

in chains of medium length: if, however, the medium becomes acid, it assumes the form of a bacillus. The length of the bacillary form varies from 1·5 μ to 3 μ or more, being greater on a solid than in a liquid medium. Figs. 3,

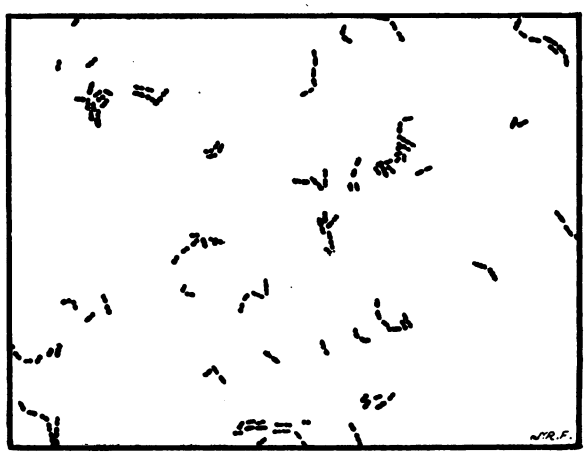

FIG. 4.—*S. mutans.* 24-hour glucose-broth culture. × 1200.

4 and 5 show the appearances of smears from 24-hour-old broth, glucose broth, and glucose agar cultures respectively.

Cultural characteristics.—It is aërobic and facultatively anaërobic. The optimum reaction for growth is about pH 7, none being obtained if the acidity of the medium is above pH 5·6. There is no growth at 22°C.

On glucose agar, small, dull, greyish-white, pin-point colonies, which seem to be imbedded in the medium, appear in about 24 hours. Seen under a low power of the microscope these colonies are opaque, brownish and round, with a lighter centre, a " hairy " texture, and a sharply-defined, usually slightly villous margin (Fig. 6). Where crowded together they remain minute, having little tendency to coalesce, and the growth has a matte appearance like ground-glass, but where more widely separated they attain a diameter of a millimetre or more in 3 or 4 days, become heaped up and irregular in outline, and are not unlike young colonies of *Actinomyces bovis* (Wolff and Israel). It is rather difficult to pick individual colonies off the surface of the medium, as they are very coherent and run along in front of the needle. Cultures on glucose agar survive for about 3 weeks. In glucose broth there is at first a general turbidity, which soon settles to the bottom as a granular deposit. Acid is very

FIG. 5.—*S. mutans*. 24-hour glucose-agar culture. × 1200.

rapidly produced, the medium, originally pH 7, giving a reaction of pH 4·2 in 24 hours; it never becomes more acid than this, however, and the organisms die out within the next 24 to 48 hours. On agar the growth resembles that on glucose agar, but is very feeble, while in broth there is a slight flocculent deposit with little or no turbidity. On Dorset's egg medium, serum media, and in fresh blood-serum a good growth is obtained, and cultures survive for a considerable time on these media as no acid is produced. On blood-agar the colonies are similar to those of other streptococci, and there is usually neither hæmolysis nor discoloration, although at times a very slight greenish colour has been observed after 48 hours. Gelatin is not liquefied.

Bio-chemical and serological reactions.—All the strains isolated ferment glucose, lactose, raffinose, mannite, inulin and salicin with production of acid; no gas; dulcite is not fermented.

An agglutinating serum was obtained by injecting a rabbit with increasing doses of a strain of *S. mutans*. This serum, which had a titre of 1 in 10,000 for its homologous strain, agglutinated all others in a dilution of 1 in 5000 or

higher; *S. salivarius*, *S. fæcalis*, *S. pyogenes* and *B. acidophilus* were not agglutinated even in a dilution of 1 in 40. Absorption tests were carried out with the homologous strain and 4 others chosen at random; the agglutinins were mutually absorbed. It may be presumed, therefore, that all strains of *S. mutans* are identical.

PRODUCTION OF ARTIFICIAL CARIES.

Three healthy teeth were sterilized in the autoclave and placed in glucose broth (pH 7) cultures of *S. mutans*. Access of the organisms to the pulp cavity was prevented by covering the roots with india-rubber teats, and the medium was changed daily so that the teeth might not remain for any length of time in an acid solution. The growth adhered markedly to the surface of the enamel, it being impossible to remove it completely by scraping or by washing under a moderately powerful stream of water. The teeth were allowed to remain in the cultures for 7, 9½ and 13 weeks respectively, and on removal they all showed considerable decalcification of the enamel, especially on the edges of the cusps and in the fissures. No naked-eye change was apparent in the dentine of the first tooth, although sections showed a commencing invasion by bacteria, but in the second and the third, brown areas were found at the bottoms of the fissures, and microscopic examination showed the dentinal tubules in these areas to be invaded by bacteria. Figs. 7 and 8 are photographs of decalcified sections of these teeth, while Figs. 9 and 10 demonstrate that the appearances seen in natural and artificial caries are practically identical, the greater dilatation of the tubules in the former being due to the fact that the decay was more advanced. In Figs. 9 and 10 the organisms seen are cocci, but the tubules in many cases appear to be filled with bacilli, both in the natural and artificial lesions.

A fourth tooth was placed in a culture of *B. acidophilus* (McIntosh, James and Lazarus-Barlow's type I), exactly the same conditions being observed. At the end of 13 weeks only a superficial decalcification of the enamel was found, and microscopic examination showed no invasion of the dentine.

It is proposed to undertake further experiments of this nature, the medium being changed automatically 5 or 6 times daily, so as to imitate mouth conditions more closely, and animal experiments are at present in progress.

SUMMARY.

B. acidophilus could be isolated only from teeth in which cavities had formed, or in which the foci of caries were shallow and contamination from the surface could not be excluded. This organism, although it will grow in a neutral or slightly alkaline medium, prefers an acid one, and would therefore find suitable pabulum in the carious cavities. *S. mutans*, on the other hand, grows best in a medium with a reaction approximating that of the saliva, is found in the earliest stages of decay, and can be isolated in pure culture from carious dentine which is more or less effectually protected from secondary infection or surface contamination.

The artificial caries experiments show that while *S. mutans* is able to

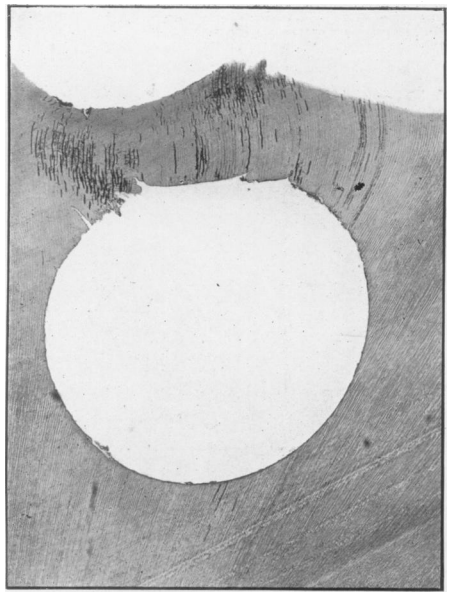

FIG. 1.
Decalcified area of carious dentine. The hole was made by the drill in taking the specimen for culture. *S. mutans* isolated. × 44.

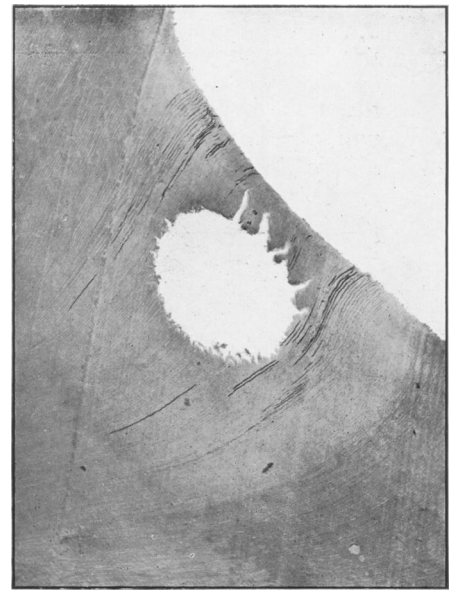

FIG. 2.
As in Fig. 1.

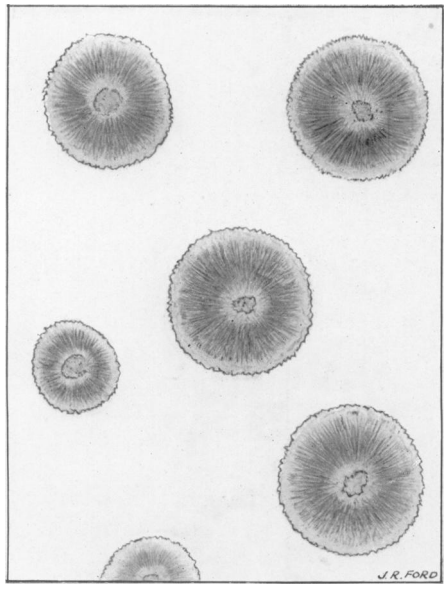

FIG. 6.
S. mutans. 24-hour colonies on glucose-agar.

FIG. 7.
Artificial caries. Decalcified section of tooth kept in cultures of *S. mutans* for 9½ weeks. × 70.

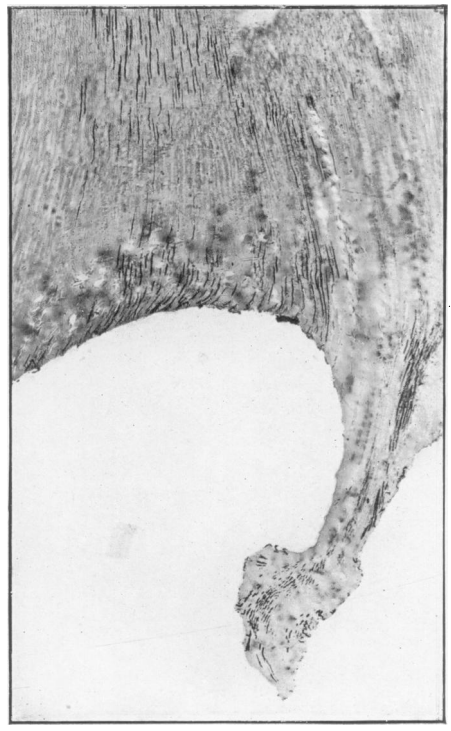

FIG. 8.
As in Fig. 7. 13 weeks.

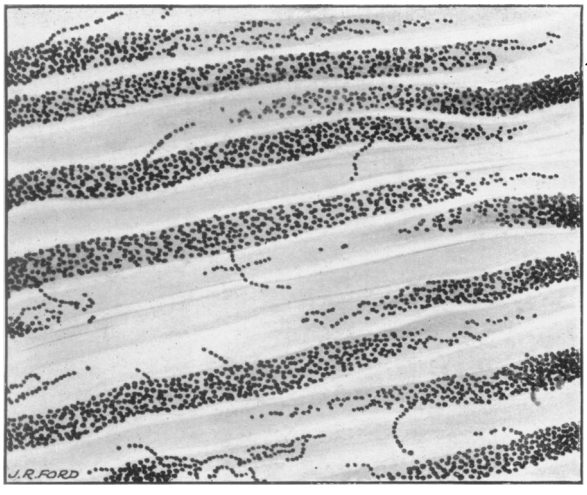

FIG. 9.
Natural caries. × 1200.

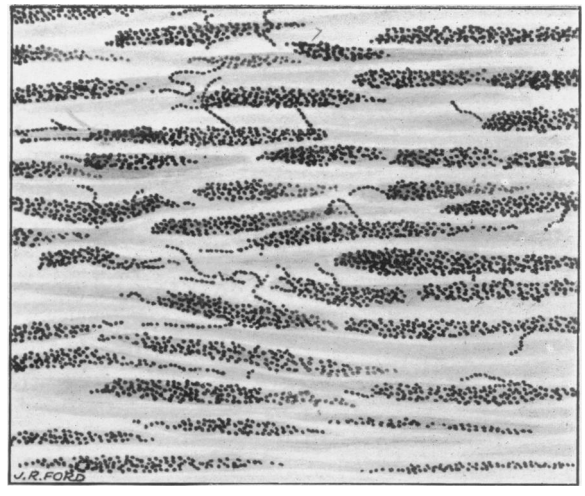

FIG. 10.
Artificial caries. Same specimen as Fig. 8. × 1200.

decalcify the enamel and penetrate into the dentine when the conditions which obtain in the mouth are to a certain extent approximated, *B. acidophilus* produces only a superficial decalcification of the enamel, which can be accounted for by the acidity of the medium. In McIntosh, James and Lazarus-Barlow's experiments the medium was changed once a week, and the teeth therefore remained in a concentration of acid sufficiently high to produce decalcification of the enamel for fully 6 out of every 7 days. The fact that the colonies of *S. mutans* adhere closely to the surface of the teeth appears to be of great importance, as a local concentration of acid in contact with the enamel is thereby produced, and this could obviously give rise to rapid decalcification independently of the acidity of the medium as a whole.

The streptococci other than *S. mutans* may be disregarded, as they are comparatively feeble acid producers, and were either entirely absent, or present only in small numbers in cases where the carious dentine was reasonably protected from secondary infection or outside contamination.

The evidence suggests that caries is due to infection of the teeth, in certain circumstances not yet made clear, by a hitherto undescribed streptococcus, *S. mutans*. Whether the primary infection is facilitated by defective structure of the teeth, such as Mrs. Mellanby (1918, 1923) has been able to produce in puppies by restricted diets, remains to be proved. Experiments in this direction are in progress.

My thanks are due to Sir Almroth Wright for extending to me the hospitality of his laboratories, to Dr. Leonard Colebrook for valuable suggestions and helpful criticism, to Messrs. Norman G. Bennett, W. H. Dolamore, A. Templar Barritt and Robert McKay for clinical information and assistance in the collection of material, to Mr. J. E. Barnard for the microphotographs, to Dr. V. D. Allison for carrying out the animal inoculation, and to Dr. P. Lazarus-Barlow for cultures of *B. acidophilus odontolyticus*.

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