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Lactobacillus acidophilus and dental caries.

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BOSTON UNIVERSITY
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Thesis

LACTOBACILLUS ACIDOPHILUS
and
DENTAL CARIES

by

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(A.B. Emmanuel College, 1949)

Submitted in partial fulfillment of the requirements for the degree of Master of Arts 1951
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INTRODUCTION

Dental caries in man has become an imminent and perplexing problem to both clinicians and research specialists. The present evidence concerning the biochemical nature of the attack on the enamel and dentin in caries is neither detailed nor conclusive. The oral microorganisms carry out many enzymatic reactions which might affect the integrity of the tooth substance. Many such reactions have been suggested as the cause of caries, such as the removal of phosphate, the removal of calcium by formation of soluble calcium complexes with hydroxypolycarboxylic acids and the removal by proteolytic enzymes of the proteins in the teeth (Kessel 1945). These reactions are, however, merely possibilities, and conclusive evidence for a primary role in dental caries is lacking.

Most of the experimental evidence presented has supported the view, first advanced by Miller (1890), that the primary attack on enamel is brought about by the action of acids in the mouth. Theoretically, any substance in contact with teeth, if it is capable of hydrolyzing calcium phosphate, that is, yielding a great enough concentration of hydrogen ions \( \text{H}^+ \) to combine with phosphate \( \text{PO}_4^{3-} \), \( \text{HPO}_4^{2-} \) and hydroxyl ions \( \text{OH}^- \) at a rate which will reduce the concentration of these ions below the solubility products for the calcium salts in the teeth, may cause decalcification if present in sufficient quantity. Bibby et al. (1942) have established that this takes place at pH 5.0 in the mouth, since in the presence of tooth substance the pH did not fall below 5.0. This, more than anything else, seems to indicate the pH at which enamel and dentin pass readily into solution, and the pH at which they exert their maximum
neutralizing power against bacterial acids. The direct relation observed between the acid formed and the amount of enamel or dentin loss indicates that this loss is entirely the result of the action of the bacterial acids.

Miller, in his studies of the bacterial flora, isolated over 100 organisms, 38 species of which he considered pathogenic. From these investigations he evolved his chemo-parasitic theory, which states that the decalcification of enamel and dentin is caused by the acids formed from carbohydrates by the action of the mixed bacterial flora. This process is followed by disintegration of the organic matrix by proteolytic action of the same or other bacteria.

Kliger (1915) reported only three organisms as significant in the caries process, *Bacillus acidophilus*, *Leptotrichia buccalis*, and *Cladotrichia placcoides*. Bunting and Parmelee (1925) presented evidence that of these organisms reported by Kliger, *Lactobacillus acidophilus*, alone, is the causative factor of the disease since this organism is found to be associated with the first lesions on the tooth in caries, and grows in high concentrations in carious mouths, while in non-carious mouths it is entirely lacking or grows in very low concentrations.

The purpose of this thesis is to review some of the studies which have been made on (1) the role of *L. acidophilus* in the etiology of dental caries, (2) caries activity tests based on these findings, and (3) possible control measures.
REVIEW OF THE LITERATURE

A. LACTOBACILLUS ACIDOPHILUS

1. Cultural and Morphological Characteristics

Lactobacillus acidophilus from the human mouth was first described by Kliger (1915) as a nonspore-forming, gram-positive, acidific, facultatively anaerobic bacillus. He listed sixty strains and emphasized the quality of pleomorphism, calling attention to the influence of anaerobiosis in the production of filaments. Bunting and Farmelee (1925), in a more extensive investigation of its characteristics, found L. acidophilus to be a slender rod 0.4 to 13 microns in length and 0.4 to 0.6 microns in breadth, which may appear in the form of cocci or long filaments, branched or unbranched. It is non-motile. In glucose and whey broth growth appears in from 2h to 48 hours. Gelatin stab cultures show a faint filiform growth with no liquefaction. The growth on glucose agar stabs is abundant and uniform from top to bottom. On glucose agar slants, a delicate streptococcus-like growth appears in 2 or 3 days at 37°C. The surface colonies on glucose agar plates are 0.5 to 1.0 mm. in diameter. They are white, smooth, slightly convex, with a regular margin and a glistening lustre. The deep colonies are of two types: (1) small, disk-shaped, compact, with even margins and no radiations, and (2) small, irregular, and possessing filamentous radiations from the center.

Bunting observed that even at the time of first isolation, these organisms manifest a highly variable morphology. No matter how many morphological varieties occurred in the original broth, however, only two types
of colonies formed when plated. Smears from many colonies of each type revealed the same range of morphological variation in the organisms, no difference being correlated with the type of the colony. If a neutral or alkaline medium was used streptococcal forms developed.

Because of the confusion arising from the pleomorphic nature of this organism, Belding and Belding (1937) published photographs of pure cultures of the organism showing that many of the coccoid forms found in the oral cavity may be transitional forms in the life cycle of L. acidophilus.

2. Evidence for a Primary Role in the Etiology of Dental Caries.

The absence of a significant difference between the solubility of enamel from carious and non-carious teeth, as established by Volker (1940), indicates that the resistance or susceptibility of a tooth to caries is not dependent on the relative acid solubility of the enamel. This finding emphasizes that a sufficiently low pH must be attained in the mouth before the caries process can be initiated. The saliva normally reaches its greatest acidity about pH 5.3 (Stephan 1948). Therefore, saliva itself cannot cause decalcification. Brawley (1935) has, however, indicated a correlation between the pH range of the saliva and the caries indices. Utilizing colorimetric pH determinations on resting saliva of 281 children, he found that with low pH ranges there were high caries indices; and with high pH ranges there were low caries indices. Since the teeth in carious and non-carious individuals do not differ in enamel solubility and the saliva is incapable of decalcifying teeth, there must be other factors which initiate the caries process.

According to Miller and others (1941), the actual exciting causes of dental caries are bacteria on the tooth surfaces capable of destroying tooth
substance by the products of their metabolism, and material on the tooth surface capable of being converted into substances harmful to the teeth. Without these two conditions, caries in all probability could not occur. All living individuals have bacteria on the tooth surfaces, and most of them, at times, have upon their teeth materials that could be utilized to form acid. Yet all teeth do not become carious. There must then be predisposing factors. These may be grouped into general factors, which exert their influence through systemic or nutritional channels, and local ones, which have their effect through the immediate environment of the tooth. Some of the general factors are age, body type, climate, emotions, endocrine activity, health, heredity, the seasons, and nutrition with all its imbalances, excesses, and deficiencies. The local or environmental influences can be grouped into four categories: (1) the effect of passage of the diet or food substances through the mouth, including consideration of the adhesiveness, the chemical composition, the detergent powers, and the particle size of foodstuffs; (2) tooth form and position, structure and substance, condition of the enamel cuticle and presence or absence of surface defects; (3) the saliva of the mouth including the amount, its alkali reserve, the presence of ammonia, antibodies, bacteriophage and lysozyme, the inorganic elements, such as calcium, phosphorus, sodium, potassium and the chlorides; the total salivary acidity and pH, the viscosity, the total solids, the mucin and other organic elements, such as carbohydrates, urea and enzymes; (4) the bacterial plaque, its bacterial content, the quantity of plaque material and its physical and chemical nature. All these factors may vary in importance in different individuals, and it is possible that their importance may fluctuate from time to time in the same individual (Kesel 1945).
Some of these factors will be taken into consideration in so far as they bear a direct relationship to the two actual exciting causes of caries.

If one or more types of acid-forming organisms are invariably found to be present at the site of the initial lesions in caries, it would appear that these organisms may be looked upon as having a specific role in the etiology of this disease. In order, therefore, to establish the lactobacilli as an initial factor in the carious process it must be shown that these organisms are capable of producing sufficient acid to cause decalcification and that they are always present in caries-active mouths.

a. Acidogenic and aciduric properties

Kliger (1915) listed as a property of *L. acidophilus* the ability to ferment glucose and lactose with avidity. Ennever (1945) has established that acid but no gas is formed from dextrose, levulose, galactose, maltose, lactose, sucrose, mannitol, sorbitol, salicin, and dextrin by *L. acidophilus* and that its final pH in glucose tomato broth generally ranges between 3.6 and 4.0.

Snyder and Teachout (1942) tested the properties of 30 strains of lactobacilli, streptococci, staphylococci, and yeast-like organisms. The lactobacilli, alone, lowered the hydrogen ion concentration below pH 5.0. In brom cresol green dextrose agar (pH 4.75), neither the streptococci nor yeast, alone or in combination, changed the color in four days. A few strains of staphylococci gave positive reactions in 48 hours. Twenty-nine of the 30 strains of lactobacilli changed the color of the medium in 48 hours. The terminal acidities in glucose broth of organisms other than lactobacilli reached the level of pH 5.0 necessary for decalcification or slightly
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TABLE 1—DENTAL CARIES

Possible Factors in Etiology or Prevention

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<th>Predisposing Factors</th>
<th>Local (Environmental)</th>
<th>Exciting Factors = Caries Activity</th>
</tr>
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<tr>
<td>General Systemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Age</td>
<td>1. Adhesiveness</td>
<td>1. Bacteria on tooth surface capable of destroying tooth substance by products of their metabolism</td>
</tr>
<tr>
<td>2. Body type</td>
<td>2. Chemical composition</td>
<td></td>
</tr>
<tr>
<td>3. Climate</td>
<td>3. Cuticle</td>
<td>2. Material at tooth surface capable of being converted into substances harmful to tooth</td>
</tr>
<tr>
<td>4. Emotions</td>
<td>4. Form</td>
<td></td>
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<td>5. Endocrines</td>
<td>5. Particle size</td>
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<td>5. Water supply</td>
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<td>7. Heredity</td>
<td>(Fluorine)</td>
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<td>(a) Acid base balance</td>
<td>(a) Density</td>
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<td>(b) Deficiencies</td>
<td>(b) Hardness</td>
<td></td>
</tr>
<tr>
<td>(c) Excesses</td>
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<td>(d) Refinement</td>
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<td>10. Food</td>
<td>6. Substance</td>
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<td>(a) Inorganic</td>
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<td>(b) Organic</td>
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<td>11. Tooth</td>
<td>7. Surface defects</td>
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<td>1. Alkali reserve</td>
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</tr>
<tr>
<td>2. Chemical composition</td>
<td>2. Amount</td>
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<td>3. Form</td>
<td>3. Ammonia</td>
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<td>4. Position</td>
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<td>6. Calcium</td>
<td>6. Physical</td>
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<td>7. Carbohydrates</td>
<td>7. Calcium</td>
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<td>11. Mucin</td>
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<td>12. pH</td>
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<td>13. Phosphorus</td>
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<td>15. Total acidity</td>
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<td>16. Total solids</td>
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<td>17. Urea</td>
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<tr>
<td>18. Viscosity</td>
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below. However, these investigators find it difficult to assume that taking into consideration the neutralizing factors in the mouth, these would have sufficient ions left for the necessary calcium exchange as compared with the lactobacilli. Bibby and McKinnon (1943) in trying to correlate acid production by salivary organisms with the total bacterial count, the streptococcal count, the lactobacillus count, or the rate of growth of these organisms, found that higher acid production was associated with higher total counts. The only relationship between the rate of bacterial growth and acid production appeared to be between the rapid growth of streptococci and rapid acid production during the initial hours of the test. Bibby et al. (1942) in another series of experiments confirmed the rapid production of acid by the streptococci. Because of this rapid acid production, they assign to the streptococci the principal role in the etiology of dental caries, basing their conclusions on the evidence of Stephan (1940) that the total glucose clearance time in the mouth is no more than 30 minutes. They also found, however, that after 24 hours the amount of acid produced by the streptococci was less than that formed by the lactobacilli, and that the lactobacilli in the presence of enamel and dentin formed considerably more acid than when no tooth substance was present. The average lowest pH values given in cultures of these organisms were lactobacilli 3.8, streptococci 4.2, actinomyces 4.5, gram-negative cocci 4.5, micrococci 4.5, leptotrichia 5.0, fusiformis 5.5, and gram-negative bacilli 7.5. Florestano (1942) felt that the presence of staphylococci and streptococci in carious mouths is as important as the presence of lactobacilli, since they have high acidogenic powers and are present in relatively large numbers. Stephan and Hemmens (1947) and Snyder and Teachout (1942) have
minimized the importance attached to the rate of acid production by these workers, since most mouth organisms produce a rapid drop in pH down to about 5.0, but only lactobacilli were found to be able to lower the hydrogen ion concentration past this critical point and to flourish in this acid medium. It is extremely doubtful if the rate of acid formation, which has been shown to be more rapid for other mouth organisms than for the lactobacilli, is as important in decalcification as the ability to live or reproduce in surroundings not conducive to survival, since a high degree of acidity must be maintained in order to complete the process. Snyder and Teachout, on the basis of a glucose broth culture tested at the end of ten days, proved the streptococci to be non-aciduric. The ability to maintain as well as produce such levels of acidity is not, therefore, a property of the streptococci. The failure of these organisms to maintain the low pH level is due to the fact that they consume acid. Stephans and Hemmens indicate that the low pH levels associated with caries activity may result not only from production of acids, but from the inability of some microorganisms further to metabolize the pyruvic and lactic acids formed. Thus oral microorganisms, such as the lactobacilli, permit an accumulation of the acid intermediates of metabolism, while other microorganisms either oxidize the acid intermediates to carbon dioxide and water or reduce them to non-acid substances such as alcohol in a relatively short time. These workers also, on determining the pH of mixtures of lactobacilli and other oral microorganisms, found that streptococci, yeasts, and sarcinae prevented the lactobacilli from reaching their lowest pH levels, which may account for the failure of Bibby et al. to correlate acid production with bacterial population curves.

Bunting and Parmelee (1925) devised a very simple experiment to show
that any disintegrating power that *L. acidophilus* may have is due to the action of its acids and not to any direct action of the organisms themselves in contact with tooth substances. To demonstrate this, they enclosed the tooth in a collodion sac with *L. acidophilus* outside. Thus the tooth was subjected to the products of fermentation and not to the direct action of the bacteria. The tooth area which was exposed decalcified.

The presence of high lactobacilli counts in some non-caries mouths, as reported by Bunting et al. (1925) and others, caused Clapper and Heatherman (1949) to conduct a series of experiments on the strain differences in oral lactobacilli. They hoped to find an explanation of the presence of these organisms in such mouths as well as to establish a better understanding of the particular properties of the bacterium that make it more or less active as a caries producer. The saliva samples used were cultured on tomato juice agar plates (pH 5.0) and the organisms transferred to peptonized milk agar. Any Gram-positive rod growing under these conditions was considered to be lactobacillus. In order to test for acidogenic properties, the organisms were transferred to tryptose broth containing glucose. The acid was titrated with n/10 NaOH and the pH was determined by means of a glass electrode after 96 hours, since longer periods produced no further lowering of the pH. Testing 148 strains, they found the lactobacilli to fall into 2 groups: (1) strong acid producers which produced a pH level of 4.1 to 4.9 and (2) weak acid producers which produced a pH level of 5.0 to 6.9.

Preliminary work with 16 strains of lactobacilli showed that the fermentation of salicin, rhamnose, raffinose and mannitol only could be used for classification of these strains. They divided the organisms into three types on the basis of these fermentation reactions as shown in Table II.
These three types were then tested for dehydrogenase activity using glucose, maltose, and fructose. Types I and III showed active dehydrogenases; Type II was less active with maltose and fructose, but more especially with glucose. Since the glucose test was the most definite, other strains were tested. Those having the biochemical activity of Type II proved to be less active than Types I and III.

The occurrence of these three types of lactobacilli in the mouths of carious and non-carious individuals was next investigated. A significantly greater number of Type I were found in carious mouths than in non-carious. These workers conclude that there is no direct correlation between acid production, in itself, and caries activity, but that by using rhamnose fermentation as a test the high acid producers can be divided into types. Examining each type for activity which would cause carious lesions, Type I was found to possess more such properties. This strain shows (1) faster growth in liquid and solid media (over III), (2) the ability to ferment more sugars (over III), (3) the ability to attain a low pH in glucose broth (over III), (4) the ability to ferment rhamnose (over II and III), and (5) active glucose dehydrogenase (over II). Type I is related to caries; Type II is related to lack of caries; and Type III bears no relation to caries. The type of lactobacilli established in the mouth is determined by some factor in the oral environment. This work has not been confirmed by other workers but it presents a possible explanation of the observed "caries immunity" in some individuals despite the presence of lactobacilli in the mouth.

aa. Dental plaques

The chief criticism of the chemo-parasitic theory advanced by Miller
TABLE II
ACID FERMENTATION BY LACTOBACILLI

<table>
<thead>
<tr>
<th>Acid</th>
<th>Type I pH below 5.0</th>
<th>Type II pH below 5.0</th>
<th>Type III pH 5.0 or above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>(except for a few strains)</td>
<td>(except for a few strains)</td>
<td>+; some strains</td>
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</tbody>
</table>

has been that it fails to explain the facts known about "susceptibility" and "immunity" to caries: and why acid formed on the tooth surface is not neutralized by the saliva. All fresh saliva within the normal ranges of pH is saturated or even to some extent supersaturated with calcium phosphate. It would seem, therefore, since the calcium phosphate of the saliva is in equilibrium with the enamel, that the enamel is thoroughly protected against dissolution by the saliva (Gore 1943). Nevertheless, these objections would be invalidated if it could be shown that the bacteria and carbohydrates produce greater acidity locally on the teeth of some than of others (Stephan 1944). Williams (1897) demonstrated the existence of bacterial plaques on the teeth, and suggested that these formations might explain the discrepancy. Plaques consist essentially of filamentous organisms which form mat-like structures. The interstices of the plaque are usually filled with bacteria and occasional epithelial cells (Hanke 1940).

Bunting and Parmelee (1925), using different strains of L. acidophilus on the entire crown of the tooth, found that there was no localization of decalcification or any evident cavitation of the enamel except that the occlusal surfaces were more whitened than the remainder of the crown of the tooth. They concluded that, in these tests, the decalcifying powers of the acids formed by lactobacillus fermentation were not adequately evaluated in that the acids formed by the lactobacilli were not confined to a given area, as in natural caries, but rather acted on a large area of the tooth. The pH value of the acids was thereby lowered. This work suggests that the bacterial plaque is essential to the carious process, since the action of the acids produced by the lactobacilli must not be neutralized by being in contact with other portions of the tooth if the maximum efficiency of the
lactobacilli is to be determined.

It is common knowledge that although decalcification of enamel occurs underneath plaques which are apparently present continuously, similar plaques appear on areas of the teeth which have undergone no visible change in structure and which may remain caries-free throughout life. Bradel and Blayney (1940) and Blayney et al. (1940) find agreement between clinical and laboratory findings which suggest that it is possible to recognize a difference in the bacterial mass recovered from either active or inactive areas. The greatest clinical activity was observed in those cases in which the smears contained either coccobacilli or short parallel rods. Non-carious plaques contained any form except short rods and coccobacilli. Studies made on the plaques of children, by means of smears and cultures, showed agreement in 83 per cent of the cases. All smears consistently showed forms of microorganisms other than the lactobacilli. These workers interpret this to mean that the caries producing bacterial plaque, which firmly adheres to the tooth surface, requires microorganisms other than the potential acid-formers. They feel that this may account for Bunting's (1925) failure to produce caries by feeding lactobacilli in milk to "immune" subjects. The microorganisms which form the plaque and are necessary for its existence are filamentous forms. They are of importance, therefore, in the etiology of caries since they form the point of localization of the lactobacilli.

Hemmens et al. (1941) made an extensive investigation of the microbial flora of the bacterial plaques isolated from carious and non-caries enamel. These organisms were grown on blood agar plates. They were tested for colony and cell morphology, staining properties, hemolytic activity, oxygen requirements, acid tolerance, and acid production. The presence or absence
of caries activity was determined by careful clinical examination and roentgenological studies. All plaques removed from areas with etching were termed carious plaques. Eighty plaques were tested; 41 from carious areas, 39 from non-carious areas. The results of their findings regarding the incidence of aciduric and non-aciduric microorganisms in bacterial plaques are tabulated in Tables III and IV. These results indicate that certain non-aciduric microorganisms, including some anaerobes, possibly represent the basic flora of the plaque. Of these only the hemolytic streptococci, of a smooth colony type, were found more frequently in carious than in non-carious areas. Of the aciduric microorganisms only the lactobacilli were recovered with greater frequency from carious areas, but these forms were not found by the methods used, in all carious plaque samples studied.

Miller, Muntz, and Bradel (1940) found that caries-free individuals possess a plaque substance which produces acid in vitro as readily as composite samples from caries-active subjects. Snyder (1942) attributes this failure to find significant differences in the formation of acid by plaque material obtained from carious and non-carious teeth to the fact that the rapid fermentation period of glucose in the material only showed the fermentation by the streptococci and did not indicate the slower acting bacilli. Stephan (1944), using an antimony electrode to measure the pH of plaques on the teeth, found a quantitative difference in the intensity and duration of the acidity produced by carbohydrates on the teeth of caries-free and caries-active individuals. The difference seems to be capable of explaining, on a chemical solubility basis, the fact that decalcification of teeth, with cavity formation, does not occur in the former individuals but does in the latter. The acid production in plaques is very rapid when carbohydrate is
<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Incidence Carious areas (39 plaques)</th>
<th>Non-carious areas (39 plaques)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Alpha hemolytic streptococci; all types</td>
<td>37*</td>
<td>95</td>
</tr>
<tr>
<td>Smooth colony</td>
<td>24</td>
<td>61</td>
</tr>
<tr>
<td>Rough colony</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Ridged colony</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Gram-positive non-hemolytic diplococci; all types</td>
<td>27</td>
<td>69</td>
</tr>
<tr>
<td>Gray-yellow</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Diptheroids</td>
<td>21</td>
<td>54</td>
</tr>
<tr>
<td>Fusiform; all types</td>
<td>21*</td>
<td>54</td>
</tr>
<tr>
<td>Flecked colony</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Gray, rough colony</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Small gram-positive rods</td>
<td>18</td>
<td>46</td>
</tr>
<tr>
<td>Micrococci, gray colony</td>
<td>18</td>
<td>46</td>
</tr>
<tr>
<td>Large gram-positive diplococci; all types</td>
<td>17*</td>
<td>43</td>
</tr>
<tr>
<td>Yellow colony</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>Gray colony</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Leptotrichia</td>
<td>17</td>
<td>44</td>
</tr>
<tr>
<td>Actinomyceses</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>Gram-positive diplococccobacillus</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Small gram-negative anaerobic cocci</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

* This figure does not represent the sum of the various subgroups but indicates the number of samples in which one or more of the various subgroups was found.
TABLE IV

INCIDENCE OF CERTAIN ACIDURIC MICROORGANISMS IN BACTERIAL PLAQUES

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Carious areas (39 plaques)</th>
<th>Non-carious areas (39 plaques)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Streptococci</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>16</td>
<td>41</td>
</tr>
<tr>
<td>Micrococci</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Yeast</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

TABLES III and IV

available and, since little initial buffering capacity must be overcome, the drop in pH is likewise very rapid. This work by Stephan shows that bacterial plaques, which are capable of producing acid, grow rapidly on the teeth of those who are caries-free. However, since a greater hydrogen ion concentration is produced in the plaques of caries-active mouths, it seems probable that these plaques differ, in some way, from the plaques in caries-free mouths. The difference is, in all probability, the presence of lactobacilli in carious plaques, since the lactobacilli were found to produce pH curves from glucose which resembled the pH curves produced by the plaques of caries-active individuals, while other microorganisms, such as streptococci and staphylococci, produced pH curves which resembled more the curves produced by plaques in caries-free individuals. Dietz (1973) invariably recovered lactobacilli and streptococci from the surface of carious lesions, the former in large numbers. Only the enamel immediately beneath the plaque is affected which, he feels, gives evidence to the fact that the more rapid rate of acid formation is essentially due to the greater number of bacteria and their species multiplicity.

Stralfors (1976), by means of an antimony electrode and an electric pH meter, investigated the change in pH of the dental plaque. At the same time he made lactobacillus counts. He found a significant statistical relation between the pH minimum and the number of lactobacilli. Individuals with a low pH minimum had a greater number of lactobacilli than those with a high pH minimum. On determining the buffer capacity of dental plaques and comparing this capacity with that of the saliva, he found that the buffering was greater in the plaque than in the saliva, especially at the lower pH values. He suggests that the plaque has the ability to store the acid and to hinder
the saliva from neutralizing it.

bb. Symbiosis of mouth organisms

Fosdick et al. (1937, 1940, 1941) and Anderson and Rettger (1937) indicate a symbiosis between lactobacilli and other mouth organisms. A culture of yeast dissolved 22 mg. of calcium per 100 ml. per 18 hours. *Lactobacillus acidophilus* under the same conditions, dissolved only 4 mg. A mixture of the two gave an average decalcification equivalent to 45 mg. of calcium per 100 ml. These workers explain the symbiosis on the basis of the phosphorylation reaction and the reductase reaction in the fermentation of glucose. Phosphorylation of the glucose is accomplished by the yeast, followed by joint production of phosphoglyceric acid, and reduction of pyruvic acid to lactic acid by the especially active lactobacilli. Since these two organisms carry out steps 1 and 4 with rapidity, but not steps 2 and 3, they suggest that other organisms in conjunction with yeast and lactobacilli may augment the rate of decalcification of tooth enamel.

Stephan and Hemmens (1947) have presented a report which contradicts these findings. They found that in pH experiments in which high concentrations of lactobacilli and yeast were mixed the acidity produced from glucose was much less than that produced by lactobacilli alone. This mixture of organisms actually prevented the lactobacilli from reaching the low pH levels associated with caries.

b. Occurrence of *L. acidophilus* in carious and non-carious mouths.

Kliger (1915) reported that lactobacilli appeared irregularly on normal teeth, but that they were present in all cases where decay had set in.
He found streptococci on all teeth. Bunting and Parmelee (1925) studying tooth scrapings, found *L. acidophilus* in every early lesion of dental caries, growing in high degrees of concentration. The methods of cultivation utilized revealed only the presence or absence of acid-forming organisms capable of cultivation on acid media and gave no information concerning the number of such organisms in the mouth from which the culture was taken, or the ability to form overgrowths on particular areas of the teeth. Direct smears from mouths of caries-active individuals showed almost pure masses of *L. acidophilus*, while similar smears from non-carious mouths contained these organisms intermingling with other mouth organisms and showing little tendency toward typical clumping or mass development.

Jay (1929) in his studies on caries in children found that it was possible to predict the onset of dental caries by a definite change in the bacterial flora of the mouth. From observations based on a 2 year bacteriologic study of a limited number of individuals, he concluded that the occurrence of dental caries is, in every case, preceded by the appearance of *L. acidophilus* in the mouth flora.

Hadley (1933) introduced a fairly accurate method of determining the lactobacillus count. Basically, this consists of culturing a stated amount of saliva on tomato juice agar, using the formula of Kulp and White (1932) with slight modifications. Using Hadley's method, the Michigan Research Group found that the relative number of lactobacilli was much higher in caries-susceptible individuals than in caries-free individuals. They obtained a 90 per cent positive correlation. (/6) In a 7 year-old group of children, Morse et al. (1936) obtained negative results for aciduric bacilli in 87 per cent of those that were caries-free, while in the caries-
active group with the highest incidence of caries, he obtained a 100 per cent positive correlation for aciduric bacilli. Blayney et al. (1939), examining 3500 specimens from 632 patients over a period of two years, found a high degree of correlation existing between the continuous presence of oral lactobacilli and dental caries, and between the absence or sporadic presence of these organisms and the absence of caries. These investigators and others (3/4, 4/7) point out that several samples, obtained within a short period of time, are necessary to confirm the presence or absence of lactobacilli in any given case. Arnold and McClure (1941) and Becks (1942) reported high lactobacillus counts in those who had active caries, while lactobacilli were absent or present in small numbers in the mouths of those in whom caries was inactive.

Boyd et al. (1935) found high lactobacillus counts in caries-free individuals, and for this reason felt that no correlation existed between the etiology of caries and lactobacilli. Anderson and Rettger (1937) reported low lactobacillus counts in caries-active individuals but high acidogenic streptococci counts. They found that, in vitro, decalcification of enamel was brought about as readily by these as by the oral lactobacilli. The explanation for this observation and the objections to assigning a primary role to the streptococci in the etiology of caries have already been cited in this paper. Speidel et al. (1939), working with diabetics, found no significant correlation between either the incidence of lactobacilli in large quantities or high acid producing capacity, with activity or inactivity of caries. Eighty-three percent of determinations on children with active caries and 60 per cent of the determinations on children free from active caries showed lactobacilli in some amount. The failure to establish a
correlation may be due to the fact that they termed caries inactive when the exposed dentin was so dense as to be impenetrable by a sharp exploring tine applied with considerable pressure. Most other investigators consider caries inactive when there is an absence of cavities or no new cavities formed within the preceding year.

Bibby (1938), after cleaning out the superficial debris, examined the bacterial flora of carious cavities. He found a low percentage of gram-positive bacilli but a great number of gram-positive cocci. He identified these organisms by the Gram stain only. A smooth strain of streptococci, isolated from carious dentin containing only coccus forms, was shown by Hammond and Tunnicliff (1940) to be able to produce artificial caries. These workers mention that they observed changes in the organism used from bacillus to streptococcus and from coccus to bacillus. This suggests the possibility that these cocci observed by Bibby and Hammond et al. are merely morphological variations of the lactobacilli, since these organisms have been shown by Bunting to be extremely pleomorphic.

Becks, Jensen, and Millar (1944) made an extensive five-year clinical survey to determine the relationship between the presence and absence of lactobacilli in caries activity and inactivity respectively. Caries activity was found to be accompanied by a positive lactobacillus index and caries inactivity by a negative lactobacillus index. In a group of 1250 rampant caries cases, 1096 or 87.7 per cent had lactobacillus indices over 1000. The reverse picture was found in 265 caries-free individuals, 218 or 82.3 per cent of whom had lactobacillus indices below 1000. This contrast between the two groups demonstrates a definite relationship. The reduction of lactobacilli by dietary measures brought
about a drastic decrease in dental caries frequency. In a group of 790 cases of rampant caries the prevention of new cavities was achieved to the extent of 80 per cent when the lactobacillus index was reduced. Of these 62.3 per cent were completely arrested, while 17.7 per cent developed only one or two cavities during the following year. This incidence of favorable response establishes the definite relationship between the reduction of the lactobacillus index and the reduction of dental caries activity.
B. FACTORS INFLUENCING LACTOBACILLUS ACIDOPHILUS

Lactobacillus acidophilus is found, in some quantity, in practically every mouth. It would appear that if this organism plays an important role in the process of tooth caries, its activity is determined not so much by its presence in the mouth as by the environmental conditions that determine the degree of its proliferation and localization in contact with tooth tissue. Blayney et al (1939) and Owen (1949), investigating the effects of orthodontic appliances and artificial dentures in the mouth, found that these increased the lactobacillus count in the mouth. These findings seem to indicate that the local environment is an important factor in determining whether or not lactobacilli will grow in the oral cavity. Of the many predisposing factors which affect dental caries, such as general physiological make-up, (5/7) endocrine activity, (5/7) prenatal and pediatric care, (5/6) and vitamin content of the diet, three factors, only, are directly related to the presence of L. acidophilus in the mouth. These are the dental plaque, the saliva, and a carbohydrate diet. The dental plaque has been previously discussed because of its effect on the acidogenic properties of the lactobacilli.

1. The effect of saliva.

Pantothenic acid and dextrose are necessary for the nutrition of the oral lactobacilli. Although the requirements for maximum growth vary with different strains of the organism, they are usually present in human saliva in optimal quantity for growth of oral lactobacilli. (Hill and Kneisner 1942). Thus the saliva will support the growth of the lactobacilli.
The reports concerning the acidity of the saliva and its relation to
dental caries are extremely contradictory. No definite conclusion can be
reached as to the exact role of the saliva in the production or neutral-
ization of acids in the mouth from the papers which have been published.
These works, however, are presented here to emphasize the possibilities
which have been investigated. Experiments performed by Volker and Pink-
erton (1947) and Hill and White (1949), on acid production in saliva in
vitro, have shown that saliva is capable of producing acidity. These ob-
servations were made on saliva-carbohydrate mixtures. The saliva samples
were obtained from carious persons. It seems reasonable to assume that the
acid was formed by the action of the lactobacilli in the saliva and not by
any chemical action of the saliva itself. Swerdlove (1942) found no cor-
relation between the incidence of dental caries and the pH of normal
resting saliva, when the acidity was determined in vivo. His experiments
were performed on 351 healthy young men.

Wills and Forbes (1936) reported that the saliva has an acid neutral-
izing power, and that the saliva and its buffers were able to dilute and
neutralize the acids which cause tooth decalcification. These investigators
reported that this power was increased by a high protein-low carbohydrate
diet and decreased by a low protein-high carbohydrate diet. Fosdick (1948)
observed that, from a chemical standpoint, there is a competition going on in
the mouth between the rate at which acids are formed and the rate at which
they are neutralized. In order to have these acids neutralized and thus
prevent decalcification by the saliva, it would be necessary to have an
abundant constant flow of highly buffered saliva to all portions of the
mouth in which acids are forming. However, mechanical impediments in the
mouth usually prevent the saliva, even if there is an abundant and constant flow, from reaching all areas of the mouth. Reed (1942) feels that the theory of an acid neutralizing power in the saliva is not tenable since the acids are nascent, the calcium is greedy for acids, the reaction is almost instant, and the plaque is a protection for the bacterial colony. Grove and Grove (1935) tried to establish ammonia or at least a nitrogenous product as the principal neutralizing factor in the saliva. White et al. (1935) investigating the ammonia content of the saliva found that this content is about the same in carious as in non-carious mouths. This evidence seems to refute the findings of Grove and Grove.

The diet, as a whole, was reported by Reed (1942) as not increasing the acidity of the saliva. He noted that an excess of acid-forming food would deplete the alkali reserve of the body and the blood reserve for this reason, would not be sufficient to clear the active gland tissues of carbon dioxide. This carbon dioxide, if retained in the gland tissues, saturates the saliva causing it to become acid. This acidity would prevent the action of ptyalin on starch, since the highest acidity tolerated by ptyalin is pH 6.8. The starch lodged in the cavity would be fermented by the lactobacilli forming acids and thus bring about decalcification. This work is corroborated by Brawley (1935) who found a correlation between the pH of the saliva and the incidence of dental caries.

The accessibility of tooth surfaces to a flow of saliva was reported by Stephan (1944) and Fosdick (1948) to be an important factor in the process of caries. Those surfaces which had relatively little access to saliva showed a greater drop in pH than those surfaces which had a relatively
great access to saliva. A slight flow not only left the bacterial colony mechanically undisturbed, but also was lacking in neutralizing power against the acids formed. Salivary currents during sleep, and the physical characteristics of precipitated mucin in the stagnant saliva might play an important role in localizing the acids formed by hydrolysis and fermentation of the carbohydrate radical in the mucin (Gore 1943). Hill (1941) reports that saliva which was thick and ropy was found to occur in carious mouths. Such saliva is below average in quantity and the lactobacilli and acids formed would not be removed as quickly by deglutition as by normal saliva.

Hill (1941), Clough (1935), and Taylor et al. (1935) found an agent in the saliva which was inhibitory to lactobacilli. The portion of the saliva that was active against the lactobacilli was filterable. It did not produce lysis but was bactericidal in effect. Hill believes that this immune factor must be liberated to the saliva from the blood. A specific factor has not been demonstrated for the lactobacilli. Ennever (1945) undertook a study to show that the saliva of a caries resistant individual does not tolerate the growth of lactobacilli because of the presence in the saliva of antibacterial factors. Each sample was used to establish a lactobacillus count and an agglutinin titer. The agglutination test used consisted of mixing the lactobacilli with the saliva sample to determine the concentration of antibody present in the saliva. Agglutination manifested itself by a cloudy turbidity which was not found in the control. The specimens were examined after 24 hours. The greatest dilution of the saliva which produced agglutination was regarded as the agglutinin titer. The results of this test are shown in Table V. Six samples were used, three from individuals with high counts, three from individuals with low counts. The results
### TABLE V

SALIVARY AGGLUTININ TITERS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lactobacillus Count</th>
<th>Agglutinin Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Less than 500</td>
<td>1:80</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1:160</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1:160</td>
</tr>
<tr>
<td>4</td>
<td>50,000</td>
<td>1:20</td>
</tr>
<tr>
<td>5</td>
<td>75,000</td>
<td>1:20</td>
</tr>
<tr>
<td>6</td>
<td>80,000</td>
<td>1:20</td>
</tr>
</tbody>
</table>

demonstrated that with each high lactobacillus count there was a correspondingly low titer of agglutination. While this antibacterial factor has been demonstrated to exist, much work has yet to be done in order to establish exactly what this factor is and how it is produced.

2. The effect of a carbohydrate diet

Miller (1891) in his chemo-parasitic theory first mentioned the role of carbohydrates as an exciting cause of dental caries. It is the carbohydrates from which the lactobacilli are able to form the acids necessary for decalcification. Bunting (1934), reporting on the work done by the Michigan Research Group, submitted evidence indicating that sugar is a very important consideration in dental caries. A remarkably low degree of caries was observed in children on a low sugar diet deficient in calcium, phosphorus, and vitamin D. However, by increasing the sugar intake, active caries was induced in these children while they were receiving a diet that was nutritionally adequate. In a majority of the individuals tested by the group, ingestion of a diet deficient in sugar was conducive to freedom from caries.

Steggard and Hill (1935), comparing the incidence of caries in Navajo and Maya Indians with that of a Michigan Dutch Group, and natives on the island of Jamaica, found that the Indians had a low incidence of caries, while the incidence of caries was high in the other groups. Price (1935), investigating caries in the relatively primitive groups on the Marquesas, Society, Cook, New Caledonia, Fiji, Samoan, and Hawaiian Islands and in the Indians of Florida, found a low incidence of caries in those living on native food, and a high incidence in those living on trade food. These workers observed that the native foods, which were not conducive to caries consisted
of small sea animals and some land plants, including fruit. The trade foods, which were conducive to caries, consisted in large part of white flour, sugar, and sweetened products. Waugh and Waugh (1936) found similar results in their investigation of caries in the Eskimo. These consume almost no carbohydrate foods and little caries occurred among them. This was true despite the fact that their mouths were filthy. These workers observed also that in over 85 per cent of these caries-free individuals lactobacilli were entirely absent.

Jay (1938) made an extensive investigation of the role of lactobacilli and a carbohydrate diet in the etiology of caries. He found that there was a low percentage of caries development on a diet which was deficient in vitamins and minerals, and free of sugar. Therefore, he concluded that caries reduction can be brought about on a diet not even suitable for normal growth and development. When large amounts of sugar, in the form of candy, were included in the diet new caries soon appeared. This increase in caries was also evident when an excess of sugar was added to a diet that was nutritionally adequate. Jay feels that the number of oral lactobacilli is proportional to the amount of carbohydrate in the diet. He was able to make the lactobacillus counts fluctuate at will by increasing and restricting the amount of carbohydrate in the diet. Occasionally, he found it necessary to resort to a diabetic diet. The caloric requirements were filled by substitution of other foods for the carbohydrates. By trial and error a diet was found on which the lactobacillus counts became negative or only sporadically positive. Caries activity was arrested in about 80 per cent of the cases by this method.

Kitchin and Permar (1948) presented evidence to bear out Jay's
findings. Carbohydrate restriction in the diet caused a reduction of the lactobacillus count in 216 cases. Boyd (1940), working with children, found that restriction of carbohydrate caused not only arrested caries, but a sclerosis of affected dentin. No sign of activity was present in the lesion. Jay (1940), conducting further investigations on the role of sugar in caries, found that some people are periodically or permanently immune to caries. More addition of sugar to the diet in these cases did not promote caries activity. On the other hand, the restriction of carbohydrates in the diet always caused a decrease in the number of lactobacilli in the mouth. Since the lactobacilli are related to the activity of dental caries, we have the distinct possibility that caries activity was reduced through the decline in the growth of lactobacilli, a result of the restriction of sugar. Becks (1942) found that carbohydrate consumption, with special regard to refined sugars, was greater in the case of caries active individuals. This, he feels, indicates a direct relationship between three factors: refined sugars, lactobacilli, and dental caries. Becks et al. (1944), in a more extensive study, found that 100 (81.7 per cent) of 1228 cases of rampant dental caries, showed a reduction of the lactobacillus index within a period of a few weeks due to the reduction of the intake of refined carbohydrates. The small remaining group manifested either insufficient cooperation or were influenced by undisclosed factors.

Stephan (1944) came to the conclusion that the presence of carbohydrates in the mouth is essential for the production of an acidity capable of decalcifying teeth, because in his experiments a pH below 5.1 was found only after the application of glucose. He felt that a significant decalcification of tooth substance would be produced by fermentation of carbo-
hydrates only when they are frequently applied to the tooth surfaces or mechanically retained on it for longer than a few minutes time. Reed (1942) agrees with Stephan on this point. His theory on the role of carbohydrates in dental caries emphasizes this agreement. He explains that starches, which are insoluble, lodge on the enamel. This lodged starch precedes and locates the bacterial colony. The bacterial colony is thus in immediate contact with the enamel. Any acid formed by the lactobacilli reacts instantly to decalcify the enamel. While sugar in solution is incapable of lodging, and thus causing a cavity, it can be used by the bacterial colony in increasing acid production, with a more rapid and destructive effect. The sugar solution needs only to be used in the bacterial set-up. Reed feels that this explains Jay's (1940) failure in some cases to produce caries by the feeding of sugar. Sugar is not an active factor in caries unless there is active starch decay, starch decay being related to the number of cavities and sugar being related to the rapidity and the amount of destruction of the teeth. Starch decay is a relatively slow process, but addition of sugar produces rampant caries. This theory, while plausible, too greatly minimizes the role of the bacterial plaque in the localization of acid. The plaque is not dependent on starch for its formation, but rather on the presence of filamentous microorganisms in the mouth. Its power to localize the bacterial colony, which forms the acid, and the acid itself has been proven by several investigators (76, 77, 78).
C. CARIES ACTIVITY TESTS

Clinical examination of the mouth does not necessarily reveal the true state of caries activity at the time of examination, but rather reveals previous caries activity. This is due to the fact that cavities may remain unchanged in the mouth for a period of time with no new lesions developing. Thus one examination which would result in a classification of caries-active might not necessarily be valid.

An activity test that is simple, accurate, and dependable would be of unlimited assistance in both research and clinical practice. Such a test would be of importance, for example, in giving a more precise classification of the caries activity of the subjects under observation. From the information thus gained, research workers would know whether the subjects from whom they were collecting material for bacterial, chemical, physical, or other analyses were caries-active or inactive at the time of collection of the material. A reliable test would also be of considerable value to research workers in studies on the prevention and control of caries. The efficiency of the measures employed could be determined in a much shorter time than the year or more which is now necessary to determine whether new cavities develop. The clinician would be aided in diagnosis and treatment planning, since it would indicate whether suspicious areas on the tooth surface should be treated or left untouched depending on the state of caries activity. In recent years, a number of tests have been developed to indicate or measure the activity of caries in the mouth. These tests are presented here along with a study of a few of these tests carried out by Kesel (1945).
1. The lactobacillus count.

The lactobacillus count, a quantitative determination of the number of lactobacilli in the mouth, was developed by Hadley (1933) on the assumption that the number of the organisms in the mouth was of greater importance than their occurrence alone. This belief has been strengthened by the experimental work of several other investigators (Hadley, 1933). The relative numbers of lactobacilli in the mouth has been offered as an explanation for the fact that a person may be caries-free and yet have lactobacilli present in the oral cavity, since if only a few of the organisms are present in the mouth, caries will not develop, due to insufficient acid production for decalcification.

The culture medium employed in this test was the tomato-peptone agar of Kulp and White (1932). Slight modifications were made because it was not possible to differentiate the lactobacilli from other mouth organisms, such as the streptococci, on the original medium. When the medium was adjusted to pH 5.0 with lactic acid, the streptococci were inhibited. An aciduric strain of streptococci sometimes appeared, but it was easily recognizable by its small size. Yeast and, occasionally, staphylococci were able to grow on this medium. However, these were easily distinguished from the lactobacilli. Smooth, intermediate, and rough strains of lactobacilli grew on this medium and were differentiated with ease. Hadley designated that the two forms of lactobacilli which produced smooth colonies should be called Types I and II, and a third intermediate form should be called Type III. The colony and cell morphology of these Types is illustrated in Figures 1 to 12. The only other modification of this medium was an increase
Figures 1 to 12

Fig. 1. Undiluted "caries-free" saliva (0.4 cc.) on glucose beef infusion agar (pH 7.0). Large colonies, yeast; small ones, streptococci, staphylococci, and B. acidophilus. Impossible to count B. acidophilus colonies.

Fig. 2. Undiluted "caries-free" saliva (0.4 cc.) same sample as in Fig. 1 on tomato-peptone agar (pH 5.0). Large colonies, yeast. Small colonies, B. acidophilus 25 per cc. of saliva.

Fig. 3. "Caries-susceptible" saliva (0.4 cc. of 1: 20 dilution) on tomato-peptone agar (pH 5.0). All colonies, B. acidophilus 25,000 per cc. of saliva. Smaller colonies, B. acidophilus, Type I: larger ones, B. acidophilus, Type II (note darker centers).

Fig. 4. B. acidophilus, Type I: organisms from Type I colony in Fig. 3.

Fig. 5. B. acidophilus, Type II: organisms from Type II colony in Fig. 3.

Fig. 6. "Caries-susceptible" saliva (0.4 cc. of 1: 20 dilution) on tomato-peptone agar (pH 5.0). Larger, white colonies B. acidophilus, Type I; smaller, gray ones B. acidophilus, Type III.
Fig. 7. B. acidophilus, Type III, organisms from Type III colony in fig. 6.

Fig. 8. "Caries-susceptible" saliva (0.1 cc. of 1:10 dilution) on tomato-peptone agar (pH 5.0). Shows yeast and B. acidophilus colonies.

Fig. 9. Undiluted "caries-free" saliva (0.4 cc.) on tomato-peptone agar (pH 5.0). Shows M. tetragenus colonies only. Note irregular borders.

Fig. 10. Patient E. R. S.; caries-susceptible; high carbohydrate diet. Saliva (0.1 cc. of 1:100 dilution) on tomato peptone agar (pH 5.0); 1,500,000 B. acidophilus, and 4,000 yeast, colonies per cc. of saliva.

Fig. 11. Patient E. R. S.; low carbohydrate diet for 12 days. Saliva (0.1 cc. of 1:5 dilution) on tomato peptone agar (pH 5.0); 4,500 B. acidophilus, and 400 yeast, colonies per cc. of saliva.

Fig. 12. Patient E. R. S.; low carbohydrate diet for 18 days. Saliva (0.1 cc. of 1:5 dilution) on tomato peptone agar (pH 5.0); 100 B. acidophilus, and 50 yeast, colonies per cc. of saliva.
in the agar content to assure solidity after autoclaving at 15 lbs. pressure for 15 minutes. The modified formula is given in detail by Jay (1938).

Tomato-peptone agar

Mixture A

1. 10 gm. of Difco peptone
2. 10 gm. of Difco peptonized milk
3. 1000 cc. tomato juice (filtered through cotton from a good grade of tomatoes.)

This mixture is heated to dissolve the peptone and peptonized milk. It is adjusted to pH 5.0 with lactic acid.

Mixture B

1. 25 gm. dried agar
2. 600 cc. distilled water

This mixture is autoclaved to dissolve the agar. Just before B is removed from the autoclave, A is brought to a boiling point. They are mixed while both are hot, and filtered through a thin layer of absorbent cotton. The result is a clear agar, light brown in color, with a final reaction of pH 5.0. Kulp's medium (pH 6.75) is available in dehydrated form at the Difco laboratories, Detroit, Michigan. Adjustment of the pH must be made, however, in order to follow Hadley's technique.

Saliva samples for the test are collected by the following procedure: the subject chews a piece of paraffin vigorously for three minutes, moving the paraffin from side to side of the mouth. This is done to assure as uniform a sample as possible. The saliva is collected in a sterile tube. The volume of the saliva is made up to 10 cc. with sterile physiological saline, so that any error caused by varying rate of flow will be minimized.
The tube is thoroughly shaken.

Dilutions of the sample are necessary in order to facilitate counting. Hadley advises a 1-5 and 1-20 dilution in sterile physiological saline for caries-susceptible cases, with subsequent dilutions of 1-100 or 1-500, if necessary, for extremely active cases. Caries-free cases, ordinarily, require no dilution of the sample. After the dilutions have been made and thoroughly shaken, 0.1 cc. from each is transferred to a hardened agar plate. In caries-free cases, 0.1 cc. is transferred to a second tomato agar plate. The inoculum is spread evenly over the surface of the agar with a sterile, bent glass rod. The plates are incubated for 3 days at 37 C., and the colonies formed counted. The final count is obtained by multiplying the number of lactobacilli counted by the dilution factor in each case.

This method was used by Hadley in a study of a group of 31 children for a period of 18 months. Fourteen of these children were caries-susceptible. The salivary samples obtained from these showed lactobacillus counts of from a few hundred to 500,000 per cc. of saliva. The average count for 79 examinations of this group was 60,000. Ten of the children were caries-free. The samples obtained from this group showed counts of from 0 to 20,000 with a majority of counts of 0. The average count for 69 examinations was 600. The other 7 children studied were not able to be classified as caries-susceptible or free. The counts made on this group were not reported. Other investigators have used this test devised by Hadley in much of the research which has been done on the role of the diet in caries and in evaluating control measures. It has been accepted by these workers as an accurate and reliable test for caries.
activity.

2. Acid production in saliva

a. Wachs' glucose fermentation test

Wach et al. (1943) developed a test which determines the ability of saliva to convert glucose to acid. They base their presentation of this as a clinical test of caries activity on the assumption that acidogenic organisms are more numerous and active in saliva from caries-active subjects. Therefore, if carbohydrate, which is fermentable, is added to the saliva, acid production quickly follows.

A 10 cc. sample of saliva is collected for the test. This sample is divided into three parts, of which Part I is used as a control. One cc. of the control is removed and titrated for total acidity with N/100 NaOH, using phenolphthalein as an indicator. A drop of the control is used to determine the pH by the use of colorimetric indicators (LaMotte). It is also possible to determine the pH with nitrazine paper (Squibb), which has a pH range of 4.5-7.5. To Parts 2 and 3 of the sample, 0.4 cc. of a 1 per cent glucose solution is added. All three tubes of saliva are incubated for 24 hours. One cc. of each sample is tested for total titratable acidity and pH at the end of 2, 4, and 24 hours. The acidity of the control and the duplicate fermentation tests are compared. Through many tests, these workers found that the 4-hour test was more critical than the one with a longer incubation period. Several tests are considered necessary before a definite conclusion can be reached concerning caries activity. An arbitrary scale has been set up to show the degree of caries indicated by this test. This
## TABLE VI

### DEGREE OF CARIES ACTIVITY

<table>
<thead>
<tr>
<th>pH</th>
<th>Total acidity per cc. of saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>7.0 - 6.0</td>
</tr>
<tr>
<td>Active +</td>
<td>5.9 - 5.5</td>
</tr>
<tr>
<td>Active ++</td>
<td>5.4 - 5.0</td>
</tr>
<tr>
<td>Active +++</td>
<td>4.9 - 4.5</td>
</tr>
<tr>
<td>Active ++++</td>
<td>4.4 - 3.9</td>
</tr>
</tbody>
</table>

- .50 cc. of N/100 acid
- .51 - .75 of N/100 acid
- .76 -1.00 of N/100 acid
- 1.0 - 1.40 of N/100 acid
- 1.41 - up of N/100 acid

scale is shown in Table VI. The test is simple, rapid, and inexpensive. Little equipment is required. It is believed that it can be used to advantage in research and dental practice.

b. Snyder's Test

Snyder (1940) devised a simple colorimetric test for the diagnosis of caries activity. This test, which determines the acid production of the saliva, was presented as a more expedient test than lactobacillus counts because it is so simple to carry out and the results are so easily read. It was presented as a more efficient test of caries activity since plate counts of lactobacilli, while distinguishing the morphological types, do not take into consideration either the acid production of the respective types or the influence exerted by other organisms on this acid production.

Saliva for the test is collected in a sterile tube over a period of three minutes time, while the subject chews paraffin. A measured amount (0.2 cc.) of the saliva is inoculated directly into a tube of sterile dextrose agar medium (pH 5.0), which contains brom cresol green as an indicator. Several trials were made by Snyder using lactose as the carbohydrate with beef infusion agar, but dextrose was found to be more reliable than lactose. The medium is bluish green in color and of a solid consistency. The color changes in the medium, after inoculation, range from no alteration of the bluish green color through stages to a distinct yellow. Snyder considered only those in which green was no longer the dominant color significant in the indication of caries activity. The test results are read as follows:

No color change in 96 hours: No activity
Positive color change in 48-96 hours: Slight activity
Positive color change in 21–48 hours: Definite activity
Positive color change in 12–24 hours: Marked activity

Snyder (1941) used this test to determine the caries activity of 63 children. The children had previously been divided into three groups: (1) a negative group of 23 children who showed neither cavities nor fillings, (2) an active group of 23 children who showed a definite increase in the number of cavities and fillings, (3) a questionable group of 17 children who showed doubtful areas or in whom there was little or no change in the number of previously recorded cavities or fillings. A close positive correlation was found between the degree of clinical caries and the caries activity as indicated by this test. Snyder and Teachout (1942) using this medium to test the acid producing powers of various microorganisms found that only the lactobacilli changed the color of the medium in 24 hours. Other organisms did not change the color of the medium even after 96 hours had elapsed. It was suggested that this test could be used to determine the relative numbers of lactobacilli in the saliva. This method of determining caries is extremely simple to carry out. Brom cresol green agar according to the formula of Snyder is available in dehydrated form at the Difco laboratories, Detroit, Michigan. No adjustments in the medium are necessary to perform this test. This test, however, follows the rule of other activity tests and several examinations at intervals are necessary to rule out chance findings.

c. Differentiation of strongly acidogenic from weakly acidogenic organisms

Davies, Slack, and Tilden (1948), following the assumption that the acid producing powers of microorganisms in the mouth determine their role in
caries, devised a medium which would quantitatively as well as qualitatively
differentiate strongly acidogenic from weakly acidogenic components of the
oral flora. The experimental medium which they employed consists of the
following ingredients:

- Tomato juice (filtered) 300 ml.
- Yeast extract 5 gm.
- Glucose 5 gm.
- Salts "A" 5 gm.
- Agar 30 gm.
- 1.6 alcoholic solution of brom cresol purple 2 ml.
- Distilled water to 1000 ml.

Salts "A" consist of 25 gm. each of monobasic and dibasic potassium
phosphate in 100 ml. of distilled water.

The medium is adjusted to pH 7.0 using the brom cresol purple as an
indicator of acidity. It is then autoclaved in 100 ml. amounts and stored
under refrigeration.

Saliva for the test is collected by chewing paraffin before breakfast
has been eaten. The saliva is then diluted 1:10,000 in nutrient broth. Be-
fore each experiment is to be performed, the medium is heated in a water bath
until it becomes fluid. To each 100 ml. of medium, 5 ml. of a sterile 8
per cent dibasic calcium phosphate solution is added and mixed by rotation
of the flask. A Petri dish is inoculated with 0.1 cc. of the saliva dilution
and when the temperature of the medium falls to approximately 45°C., 20 ml.
are poured into the dish. The plate is rotated quickly to insure uniform
distribution of the saliva sample. All plates are incubated at 37°C. for
four days.

The colonies are distinguished by the following reactions:

1. Strongly acidogenic colonies dissolve the calcium phosphate forming a
halo 3 mm. or more in diameter. The color of the medium is changed from
purple to yellow.

2. Moderately acidogenic colonies form a smaller halo than those of (1). The color is also changed from purple to yellow.

3. Weakly acidogenic colonies do not dissolve the calcium phosphate but do produce the color change as above.

4. Neutral colonies produced no change in the medium.

5. Alkali producing colonies are surrounded by a zone of deep purple color. There is no dissolution of the calcium phosphate.

The dissolution of the calcium phosphate takes place at pH 4.0. The color change from purple to yellow takes place at pH 5.35 to 4.5.

In order to evaluate this medium as a test for caries activity these workers tested 87 samples of saliva. As controls, tomato agar plates, Snyder's medium, and glucose broth of an initial pH of 6.2 were inoculated. The results were compared with clinical data, and a fair correlation was obtained. This medium, however, while it has a possible use as a test for caries activity is very difficult and time consuming to prepare. Even if its reliability and accuracy were proven beyond a doubt, it would not be expedient for use either by the clinician or research worker since the tests which have been previously presented are as reliable and are much easier to perform.

d. Rhamnose fermentation test

Clapper and Weatherman (1949), in their studies on strain differences in lactobacilli, found that their Type I lactobacilli, which fermented rhamnose, were more numerous in carious mouths than in non-caries mouths. More fermentation tests were carried out by these workers by inoculating rhamnose
with saliva samples. They found that rhamnose fermentation had as high a correlation with caries as did the positive identification of Type I lactobacilli. They suggest that a positive rhamnose fermentation test may indicate caries when only a few organisms are present. Their work on the lactobacilli has not been confirmed, nor have any experiments been carried out to determine the efficiency of rhamnose fermentation as a test for caries activity. However, if the test could be proven reliable and accurate, it would be a simple and inexpensive test for clinicians and research workers.

3. Enamel dissolution test

Fosdick, Hansen, and Epple (1937), in attempting to determine the solubility of human enamel in saliva in the presence of sugar, developed a test which they suggested would be useful in indicating caries activity. This test consists in suspending powdered enamel in a mixture of 6 cc. of saliva, stimulated by chewing gum or paraffin, and 0.2 gm. of glucose. This suspension is agitated in a water bath of 37°C. for four hours. The sample is then centrifuged to remove the dissolved enamel. The difference between the original calcium content of the saliva and the calcium content after the experiment was taken as a measure of the solvent action of fresh saliva on the human enamel. This experiment was performed on saliva from mouths of both susceptible and immune subjects by these investigators. Enamel dissolution increases above 9-10 mg. were reported as increases in caries activity.

4. Study of the dental plaque

a. Microscopic examination of plaque material

This method was devised by Blayney, Kesel, and Wach (1936). Plaques,
which were carefully removed from the tooth surfaces under observation, were stained and examined microscopically. A great number of gram-positive coccobacilli were observed in plaques from caries active surfaces, while a mixed bacterial flora of a different nature was observed in plaques from caries free areas. The disadvantage of this test is that no indication is given of the degree of caries activity present.

b. Determination of the pH of plaque material

Stephan (1938), using an antimony electrode found that the dental plaques in carious areas of the mouth differed in pH value from those in non-carious areas. The acidity of the "carious plaques" was found to be much greater than that of "non-carious". This test, however, does not take into consideration the various factors in the mouth, other than the dental plaque, which influence the production of acid by lactobacilli. This is also true of the previous test described, that of direct microscopic examination of plaque material.

5. Evaluation of some activity tests

Kesel (1945) conducted a study on the accuracy and validity of a few of the activity tests previously described. Fifty subjects were used in this study, each of whom was given two careful mouth examinations annually with complete full mouth roentgenograms. The study had been in progress for two years at the time of Kesel's report. The tests which were studied were (1) the lactobacillus count according to the technique of Hadley, (2) the glucose fermentation test as devised by Wach, and (3) the enamel dissolution test according to the method advanced by Fosdick and Hansen.
The experiment was set up to determine the ability of each of the tests to detect caries activity. It was also the objective of the investigator to discover whether or not the results of the tests could be correlated with clinical activity, that is, with the approximate number of new cavities developed and the increase in the size of the existing cavities. Arbitrary standards were set up to indicate the results of the activity tests and for measuring clinical activity. These standards are shown in Tables VII to X. It was noted in this study that 90 per cent of the lactobacillus counts, 94 per cent of the enamel dissolution tests, and 85 per cent of the glucose fermentation tests indicated correctly the trend of caries activity in the subjects used. The enamel dissolution and glucose fermentation tests, in many of the cases, indicated a greater activity than was observed clinically. The glucose fermentation test, especially, tended to overestimate caries activity according to the standards used to judge it.

The three were found to be largely dependable as to indicating whether caries was active or inactive, but were not so accurate in indicating the degree or amount of activity in the mouth over a long period of time. It was suggested that several tests be made, and an average or trend determined before a case is classified as active or inactive when using any of the methods described. The reason for this suggestion is evidenced by results obtained in the lactobacillus counts. Most of the inactive cases showed no growth on the tomato agar plates, while those that had active caries gave counts on these plates that were in the thousands. However, in one inactive case three tests gave high counts which would place it in the active group, although thirteen of the cultures were negative.
TABLES VII to X

### TABLE VII
STANDARD FOR CLASSIFYING CLINICAL ACTIVITY

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>No new lesions and no increase in extent of questionable areas</td>
</tr>
<tr>
<td>Active+</td>
<td>1-2 new lesions</td>
</tr>
<tr>
<td>Active++</td>
<td>3-5 new lesions</td>
</tr>
<tr>
<td>Active+++</td>
<td>6-10 new lesions</td>
</tr>
<tr>
<td>Active++++</td>
<td>11 or more new lesions</td>
</tr>
</tbody>
</table>

### TABLE VIII
STANDARD FOR CLASSIFYING LACTOBACILLUS COUNTS

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>No lactobacillus colonies to 100</td>
</tr>
<tr>
<td>+:</td>
<td>More than 100 colonies to 10,000</td>
</tr>
<tr>
<td>++:</td>
<td>More than 10,000 colonies to 100,000</td>
</tr>
<tr>
<td>++++:</td>
<td>More than 100,000 colonies to 1,000,000</td>
</tr>
<tr>
<td>++++:+</td>
<td>More than 1,000,000</td>
</tr>
</tbody>
</table>

### TABLE IX
STANDARD FOR CLASSIFYING ENAMEL DISSOLUTION TESTS

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>0 to 5 mg. calcium increase</td>
</tr>
<tr>
<td>+:</td>
<td>More than 5 mg. to 10 mg.</td>
</tr>
<tr>
<td>++:</td>
<td>More than 10 mg. to 20 mg.</td>
</tr>
<tr>
<td>+++:</td>
<td>More than 20 mg. to 30 mg.</td>
</tr>
<tr>
<td>++++:+</td>
<td>More than 30 mg.</td>
</tr>
</tbody>
</table>
TABLE X

STANDARD FOR CLASSIFYING ACID FORMATION TESTS

<table>
<thead>
<tr>
<th>PH</th>
<th>Total acidity per cubic centimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>7.0 - 6.0</td>
</tr>
<tr>
<td>+</td>
<td>pH drop to between 5.9 and 5.5</td>
</tr>
<tr>
<td>++</td>
<td>pH drop to between 5.4 and 5.0</td>
</tr>
<tr>
<td>+++</td>
<td>pH drop to between 4.9 and 4.5</td>
</tr>
<tr>
<td>++++</td>
<td>pH drop to between 4.4 and 3.0</td>
</tr>
</tbody>
</table>
D. CARIES CONTROL MEASURES

The control of dental caries has become a major health problem. Caries cases have increased by a tremendous percentage in the last fifty years. The hope of combatting this problem lies not in reparative dentistry, but in some adequate method of prevention. Since caries is the result of acid decalcification of the enamel, it could possibly be prevented (1) by making tooth substance resistant to decalcification, (2) by preventing the formation of acids on the tooth surface, or (3) by neutralizing or buffering the acids before they can attack the enamel. Several methods have been advanced which attempt to perform at least one of these functions.

1. Making the tooth resistant to decalcification

The possibility of achieving a method of making the tooth resistant to decalcification is extremely remote, since the tooth substance is composed predominantly of calcium phosphate and, as such, is soluble in lactic and similar acids.

a. Fluoride treatment

It was suggested by Dean (1941) that, since a negative correlation prevailed between the fluoride concentration in the public water supply and dental caries, fluorides in or on the enamel might make the tooth caries resistant. The action of fluorides in lowering caries incidence is not fully understood. It is known, however, that (1) fluoride is incorporated into the hydroxy-apatite structure of the enamel with facility, resulting in an enamel of greater hardness and less solubility to acid, and (2)
fluoride is an effective inhibitor of several enzyme reactions, especially those concerned with carbohydrate metabolism of the bacterial cell (Nuckolls and Frisbie 1946). Although the possible role of fluorides in reducing caries incidence may be in making the tooth resistant to decalcification, as suggested by Dean, the work of Miller (1938), Dale et al. (1944), Retarski et al. (1945), and Jay (1946) gives precedence to their action as enzyme inhibitors. The evidence presented by these workers is strong enough to warrant the consideration of fluorides as factors which prevent fermentation. For this reason, a discussion of fluoride treatment in the control of caries will be considered with these factors.

b. Silver nitrate treatment

The topical application of silver nitrate was advocated by Zander (1941) as a possible means of reducing caries. This method was presented on the assumption that metallic silver, if precipitated into the area of the tooth affected by caries, would serve as a barrier to the further progression of caries in the region. The antibacterial properties of metallic silver felt to be the inhibitory factor. This method was found to be of no practical use, however, by Klein and Knutson (1942). In a study of 474 children, they found that teeth treated with silver nitrate decayed at about the same rate as teeth that were not treated, and that the original carious condition extended to about the same degree in both groups.

2. Prevention of acid formation

It has been established that the acids which cause decalcification of tooth substance result from the action of lactobacilli on carbohydrate
material. It should, therefore, be possible to prevent the formation of these acids (1) by eliminating the lactobacilli, (2) by materially reducing the fermentable carbohydrates, or (3) by inhibiting the action of bacteria on the carbohydrate material. If any of these three could be successfully accomplished, dental caries could be prevented.

a. Immunologic reactions

Many persons, who are "immune" to caries, demonstrate a resistance, possibly due to an immunological principle, inhibitory substance, or antibody acting through the blood stream or saliva. This is a real possibility, since no other theory has been definitely proven to explain this "immunity".

The Michigan Research Group (1933) became interested in determining tests for susceptibility to dental caries. A filtrate was made from culture of lactobacilli, and this filtrate was used in skin tests to determine what the reaction would indicate as far as susceptibility to caries was concerned. They found a positive reaction to the test indicated lack of lactobacillus antibodies in the blood serum, while a negative reaction indicated the presence of antibodies in the serum. Presence or absence of antibodies was determined by using the agglutination test. When these findings were correlated with the incidence of caries, it was found that persons with antibodies in their sera were immune to caries, and lactobacilli were not present in their saliva samples. These workers then prepared cultures of lactobacilli for injections to determine if antibodies could be stimulated. The suspensions prepared for the injections included a rough and smooth strain of the lactobacillus. Subcutaneous injections were made. The rough strain of the organism was found to cause sterile abcesses in the tissues,
but the smooth strain did not produce any reaction. Blood sera, obtained at this time, were measured for antibodies by the agglutination test. The sera from persons with abscesses had an increased antibody concentration against lactobacilli, while that from those with no abscesses showed no appreciable change in lactobacillus antibody concentration. These investigators were not able to determine whether these injections and the antibodies produced had any effect on the lactobacilli in the mouth.

Canby and Bernier (1942) conducted a study to determine what the effect of increased antibody in the bloodstream might have on the lactobacilli in the mouth. They selected certain strains of lactobacilli for the injection by the process of cross-agglutination tests. The tests were conducted to find a strain of lactobacillus that could stimulate antibodies against any of the others. Since they found marked heterogeneity among the lactobacilli, no attempt was made to classify the organisms in groups or types. Each strain was tested separately. Intradermal injections were used in the experiment instead of subcutaneous, even though the subcutaneous injection is the preferable method for stimulating antibodies. Lactobacillus counts were made on the subjects previous to and 4 to 10 days after the injections. In almost every instance the average number of lactobacilli in the mouth was decreased as a result of the injections and consequent antibody formation.

Williams et al. (1944) followed up these experiments, adding a group of controls. There was one change in procedure, that of giving subcutaneous rather than intradermal injections. All subjects were bled to obtain serum. Injections were given at weekly intervals for 4 weeks. Into one arm a living suspension of a mixture of two lactobacilli was injected, while a
heat killed suspension of the same mixture was injected into the other arm. Salivary counts and sera were obtained at regular intervals from the injection and control groups, beginning two weeks after the last injection and continuing through five months. Agglutination reactions were used to test the sera. The highest agglutinin titers were found two weeks after the last injection. These titers had reached an approximately "normal" level at the end of five months. A statistical analysis of the lactobacillus counts indicated that 75 per cent of the immunized had reductions in average salivary counts. Twenty-five per cent of the group had reductions which were so slight that they were regarded as chance. The results obtained did not indicate that the immunization, as performed, had any effect, consistently, on the numbers of lactobacilli in the mouth. They felt that the strains of lactobacilli used might not have been the best ones for immunization.

The work which these investigators have done on immunization to dental caries is not conclusive. However, with further studies along these lines, it may be possible to prepare a vaccine which will be successful in stimulating enough antibodies to obliterate the lactobacilli of the oral cavity. The implications of the discovery of such a vaccine in caries control are evident.

b. Carbohydrate control

Jay (1947) studied the effect of restriction of carbohydrates on lactobacillus counts in the mouth. His plan cut consumption of carbohydrates to 100 grams daily. Carbohydrates were gradually added to the diet over a period of time until a point was reached where the diet was again unrestricted.
On the restricted diet, the lactobacillus count was reduced in over 80 percent of the cases studied. Becks, Jensen, and Millar (1944) had previously established that reduction of the lactobacillus count was obtained by restriction of carbohydrate. Control of the carbohydrate in the diet would serve two purposes: (1) reducing the media necessary for bacterial growth and (2) reducing the material which is fermented to form the acids necessary for decalcification. The only method of preventing carbohydrates from reaching the vulnerable areas of the teeth is their elimination or material reduction in the food consumed daily. The fact that a low carbohydrate diet is conducive to a low incidence of caries has been noted by several investigators, previously cited, while determining the caries incidence in primitive groups. However, while such control of the diet would undoubtedly be a striking preventive measure in caries control, the appetite for carbohydrate food is firmly entrenched in most countries today. Cooperation with regard to dietary restriction of carbohydrates would be almost impossible to obtain. For this reason, attempts to prevent dental caries by widespread dietary control would not be practical.

c. Use of penicillin

Hill (1948) suggested that, because of its antibiotic properties, penicillin might be effective in reducing the lactobacillus count in the oral cavity. He performed an experiment, using 10 well selected subjects, on the effect of a penicillin dentrifice. At the beginning of the experiment these subjects had an average lactobacillus count of approximately 72,000 per cc. of saliva. At the end of one week the count dropped to 18,000 and kept on dropping for 5 months until it reached a count of 300. The subjects con-
continued to use the penicillin dentrifice for another week and then began to use commercial dentrifices. The lactobacillus count remained at an average of 300 for three months, and during the fourth month returned to approximately the 72,000 per cc. of saliva which it had been originally. After the subjects again began to use the penicillin dentrifice the counts dropped, as in the first test, but much more slowly. The sensitivity of various strains of lactobacilli to penicillin was found to be variable. This was determined by using a well formation in the center of an agar plate. Lactobacilli were placed in the well; the penicillin in the surrounding medium. The effect of the penicillin was easily evident. The penicillin dentrifice experiment was carried out a second time, using a group of boys 7 to 14 years of age. They did not prove to be a cooperative group. Hill points out that the results indicate what might happen if penicillin medication was given to the general public rather than a selected group. The subjects were instructed to brush their teeth once daily with the penicillin dentrifice. After a five month period, it was found that in the control group 21 per cent had increased lactobacillus counts and 43 per cent had decreased counts. In the experimental group, only 1 per cent had increased lactobacillus counts and 65 per cent had decreased counts. It was not possible at the time of Hill's report to determine whether or not there had been a reduction in caries incidence.

White et al. (1949), also studying the effects of penicillin on the oral flora, found that the number of oral lactobacilli can be reduced by the proper and conscientious use of penicillin dentrifice. It was noted that the balance of the natural bacterial flora of the mouth was changed little by small amounts of penicillin, but that larger doses brought about a change in balance, evidently to replace that part of the flora destroyed by penicillin.
They found that this change in the balance of the flora was not maintained, however. In these investigations by Hill and White, no allergic reactions to the penicillin were manifested. It is believed that a few individuals would possibly be allergic to this antibiotic, but such allergy would not be dangerous if manifested.

3. Prevention of fermentation

Since no definite means has been proven completely reliable for removing either the lactobacilli or the fermentable carbohydrates in the mouth, several attempts have been made to determine a method for preventing the action of bacteria on the carbohydrates, or for neutralizing the acids formed before they can attack the enamel. Stephan (1950) has shown that acid production begins almost immediately after carbohydrates are ingested, and that total glucose clearance time in the mouth is approximately 30 minutes. Thus any method which is designed to prevent fermentation or to cause neutralization of acids must be used a very short time after the ingestion of carbohydrates.

a. Mechanical cleansing of the teeth

For many years the slogan, "A clean tooth does not decay.", has been a byword in dental practice. Mechanical means for cleansing the teeth have been developed in an attempt to achieve "clean" teeth. Prophylaxis, which consists of routine scaling and polishing of the teeth by the dentist every 3 to 6 months, has been suggested as having a possible value in the prevention of caries. However, Hine and Bibby (1939) were able to obtain bacterial plaques by use of a scaler from the same area of the tooth 16 times in
18 days. Since the plaques are able to form again in such a short time, prophylaxis is of questionable value in caries prevention. The value of such procedure would possibly lie in the smoothing of rough areas, which would, in some measure, prevent the retention of food. No complete studies on the efficiency of prophylaxis in caries control are available.

Toothbrushing, in order to be effective, must remove the food debris immediately, and must be done thoroughly. Since it is inconvenient, usually, to brush the teeth immediately after eating, this practice has not achieved any prominence in the control of caries. Not only are the teeth not brushed soon enough, but the ordinary person does not take sufficient time, or use sufficient energy, to do a thorough job. Fosdick (1942) reported the results of a study on the effect of toothbrushing immediately after eating. Twenty caries-active patients were used in the study. All cavities were filled. These patients used an antiseptic rinse immediately after the ingestion of carbohydrate food; brushed the teeth with a good brush and a dentrifice; and then chewed a stick of antiseptic paraffin. The investigator reported an appreciable decrease in the susceptibility of every patient in the test. However, conclusions cannot be drawn from an uncontrolled study of such a small group. Fosdick now has over a thousand persons using this method, but no report has been made concerning this study. One benefit which may be obtained from brushing the teeth, as from prophylaxis, is the polishing and smoothing of the tooth surface. This would prevent, in some measure, the lodging of food and bacterial plaques. The use of dental floss, toothpicks, and various gadgets, designed to clean interproximal surfaces, should be of value in removing impacted food debris, but no work has been done to prove that they are of any value in caries control.
While all of these mechanical methods, as well as the use of non-medicated mouth rinses and detergent foods, have an esthetic value as cleansing agents, a definite influence in the control of caries has not been established. Any one could be only partially successful in removing oral bacteria and, in most instances, food debris, and then even this partial effect would be only temporary. For this reason, it is felt that these measures could at best only slightly reduce dental caries.

b. Use of chemical agents

Several reports have been made in the last few years on the use of different chemical agents to check the action of acidogenic bacteria in the oral cavity. These chemicals are usually incorporated in mouth rinses. Hanke (1940) tested several agents in search of an oral antiseptic that would destroy plaques and reduce or eliminate acid formation on the tooth surface. The organic mercurials were found to be superior in this regard. However, most of the organic mercurials tested were observed to have such undesirable effects as discoloration of the teeth, amalgamation with gold fillings, and a metallic taste. He finally discovered one organic mercurial, sodium p-hydroxymercuribenzoate, which could be compounded into a mouthwash that did not have the above mentioned undesirable effects. The name Solution 58 was given to this mouthwash. He feels that the solution is of value in preventing fermentation, since it evidently destroys the cohesive and adhesive properties of the filamentous organisms which form the basis of the plaque. This solution was tested by use in 95 cases. It was noted that these persons, using Solution 58 once or twice a day, did not have plaques on the teeth. Specimens of saliva, which were collected
2, 4, and sometimes 9 hours after rinsing the mouth with this solution, produced little or no acid when incubated for 4 hours in the presence of sugar. Hanke believed, therefore, that it was possible to cause destruction or inhibition of acidogenic organisms with this solution so that they were no longer significant. Use of the solution caused dental caries to be arrested in fifteen patients who were highly susceptible to the disease.

The topical application of fluorides and the use of fluorides in drinking water has been suggested by several investigators. Dean et al. (1941), investigating domestic waters, found a negative correlation between the fluoride concentration of the water and dental caries. It was noted that the differences in the lactobacillus counts in the saliva corresponded to the differences in dental caries experience. In this case the fluoride was inhibitory at a low concentration (1.2 p.p.m.) so that mottled enamel was not a problem. Arnold et al. (1942) found that treatment with water containing a fluoride concentration of from 0.1 p.p.m. to 0.7 p.p.m. resulted in little if any decrease in caries activity. This is not surprising, since Clapper (1947) found that 1 p.p.m. of sodium fluoride had no demonstrable effect on lactobacilli in vitro. He noted, however, that continued exposure to 100 p.p.m. sodium fluoride lowered the ability of an actively acidogenic strain of lactobacillus to produce acid from glucose. This reduced acidogenic activity continued in the absence of sodium fluoride. Jay (1946) observed that fewer cavities were found in areas with fluoride in the water and also, that the lactobacillus counts were lowered. This bears out the work of Dean. Jay felt that this work might possibly justify the topical application of fluorides on the teeth, since it seems to inhibit the enzymes concerned with acid formation on the tooth surface. There may be
other chemicals which could be of more use in this manner in dental caries control, but to date the fluorides alone have been investigated to any extent. They have been placed in the water supply of many cities and topical application on the teeth of school children is now common. Some time will be necessary before the effectiveness of fluoride treatment can be fully evaluated.

The use of urea as a means of controlling caries was suggested by Stephan (1940). He showed that, in addition to the secreted buffers, the urea in the saliva could be converted to ammonium carbonate by urease-producing bacteria on the tooth surface. This ammonium carbonate is alkaline, and would act as a buffer or neutralizer for the acids of fermentation. The ability of urease-producing bacteria to convert urea to a buffer for acids has been established by Hine and O'Donnell (1943). This concurs with the work of Stephan. Since ammonia acts as a buffer or neutralizer for the acids in the mouth, although its effectiveness has not been evaluated, Kesel et al. (1943) suggested the use of an ammonia containing dentrifice as a means of caries control. Such dentrifices are now being sold in commercial preparations, but, as in the case of fluoride treatment, they have not been fully evaluated.

Wach, O'Donnell and Hine (1942) reported on the effectiveness of a solution of urea and quinine in preventing acid formation. They believed that the action of the rinse they suggested depended on both components. According to their theory the urea becomes alkaline and a buffer through its conversion to ammonium carbonate by urease-producing bacteria. Quinine not only prevents lactic and butyric acid production, but also has bactericidal power. The effectiveness of quinine is greater in an alkaline
medium, and thus the alkalinity produced by the ammonium carbonate enhances the bactericidal efficiency of quinine. This rinse was tested and a reduction in the lactobacillus counts and in the ability of the saliva to convert glucose to acid was observed. Clinical tests have not yet been made. Possibly such tests might prove this rinse to be of value in caries control.
SUMMARY AND CONCLUSIONS

There are several predisposing factors in the etiology of dental caries, among which are age, body type, climate, emotions, endocrine activity, health, heredity, the seasons, and the nutrition of the individual. Resistance or susceptibility of a tooth to caries is not dependent on the relative acid solubility of the enamel, since caries-resistant teeth are as readily soluble in acid as those which are caries-susceptible. It has been established that a pH of 5.0 or less is necessary to bring about enamel dissolution. Saliva does not reach this level of acidity and, therefore, cannot be responsible for tooth decalcification.

The actual exciting cause of dental caries is the presence of microorganisms on the tooth surface capable of producing metabolic products which will destroy tooth substance, along with material on the tooth surface capable of being converted into substances harmful to the teeth. The available evidence indicates that *L. acidophilus* is the microorganism which has a primary role in the etiology of dental caries, since its nature is sufficiently acidogenic and aciduric, and it occurs in demonstrable quantities in over 90 per cent of carious mouths. Reports to the contrary may be due to incorrect identification of the organism due to the pleomorphic nature of the lactobacilli. Staphylococci, streptococci, and yeasts, while they are as acidogenic as the lactobacilli, are not sufficiently aciduric to assume a primary role in the etiology of caries. This disintegrating power of *L. acidophilus* on tooth substance is due to the acids produced by the organism and not to any direct action of the organism itself. The evidence also indicates that the material on which the lacto-
bacilli work is the carbohydrate of the diet.

The resistance of some individuals to caries even though lactobacilli are present in the oral cavity has led to the investigation of strain differences in lactobacilli. There is a possibility that the lactobacilli may fall into groups which differ in their acid producing powers and their relation to caries. The presence of lactobacilli in some non-caries mouths may be explained by these variations. It has also been suggested that this "caries immunity" may be due to the relative numbers of lactobacilli in the mouth, since a small number of lactobacilli would not produce sufficient acid to cause decalcification. A symbiosis may also exist between the lactobacilli, yeasts, and other mouth organisms. However, sufficient evidence is not available to either establish such a symbiosis or disprove it.

The activity of *L. acidophilus* is affected by the dental plaque, the saliva, and by a carbohydrate diet. Of these, saliva exerts the least effect. Normal saliva contains the nutritional requirements for lactobacilli and will support their growth. No definite conclusions can be reached as to the exact role of the saliva in the production or neutralization of acids in the mouth. An agent exists in the saliva, however, which is inhibitory to lactobacilli. Its exact nature is still unknown. The dental plaque and the carbohydrate of the diet, on the other hand, have a marked effect on the activity of lactobacilli. Bacterial plaques are formed by filamentous bacteria. They are essential to the carious process since they form a point of localization of the lactobacilli and protect the acids produced from being neutralized by the saliva. All carious plaques contain lactobacilli, non-caries plaques do not. The hydrogen ion concentration
produced by plaques from carious teeth is greater than that produced by plaques from non-carious teeth, because of the presence of these lactobacilli. Caries activity is directly proportional to the carbohydrate intake of the diet, since carbohydrate is essential for the production of acidity capable of decalcifying teeth.

Several caries activity tests have been formulated and tested. The tests of Hadley (the lactobacillus count), Wach (glucose fermentation test), Snyder (colorimetric test), and Fosdick (enamel dissolution test) show a positive correlation between test results and clinical activity of caries. These tests are the most reliable of those reported. Their reliability, however, depends on the test being made several times in each case.

A method for controlling caries has not yet been established although many have been suggested. Treatment with silver nitrate or fluoride to make tooth substance resistant to decalcification has not proven successful. Carbohydrate control has been proven reliable in controlling caries, but widespread carbohydrate control would be impractical. Much experimental work has been performed on the use of penicillin and on immunologic reactions both of which would prevent fermentation by destroying the lactobacilli. These experiments have had positive results up to the point which they have been carried. The same is true of experimental work on the use of organic mercurials and fluorides to neutralize or buffer the acids, prevent enzyme activity, or hinder the formation of the plaque before decalcification is accomplished. Further work on these control measures is now in process and the possibility exists that one of these may be the much desired and sought after measure which will bring about the control of dental caries.
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ABSTRACT

The etiology of dental caries is a problem which has for many years absorbed the attention of a number of research workers. Several theories have been formulated in an attempt to establish the actual causative factor. The theory which best seems to offer an explanation is that microorganisms are present on the tooth surfaces which are capable of forming metabolic products from the foodstuffs there and that these products bring about the dissolution of the enamel. This theory was first advanced by Miller (1890).

The question then arose as to which microorganism in the mouth was capable of this action. Kliger (1915) observed a high percentage of Lactobacillus acidophilus in carious mouths and, since that time, several other workers, including Bunting and Parmelee (1925) have advanced evidence that this organism is the actual exciting cause of dental caries. The lactobacilli are highly pleomorphic in nature and are sufficiently acidogenic and, possibly more important, aciduric to cause dissolution of the enamel. No other organism in the mouth has been shown to have these essential qualities. In support of this evidence, Bunting et al. (1925), Jay (1929), Hadley (1933), and Becks et al. have shown that a positive correlation exists between the number of lactobacilli in the mouth and the incidence of dental caries.

These lactobacilli do not exert their action independently of the other components of the oral cavity. It is possible that the saliva has some effect on their action, but this has yet to be proven. The bacterial plaque, on the other hand, has been established as necessary for the carious process. The organisms which compose the plaque form a filamentous mesh-like structure which localizes the bacteria and the acids which they form.
Bradel and Blayney (1960) have reported that microscopic examination of plaque material from carious areas on the teeth showed great numbers of lactobacilli while those from non-carious areas did not. Equal in importance to the bacterial plaque is the carbohydrate of the diet. This carbohydrate is the material from which the lactobacilli form the acids causing decalcification. Becks et al. (1944) and Jay (1947), along with others, have established the importance of carbohydrate in their studies. In all cases they found that a high carbohydrate content in the diet was associated with high caries incidence, and that low carbohydrate was associated with low caries incidence. Carrying these studies further they found that reducing the carbohydrate of the diet materially reduced the caries incidence in susceptible cases.

Since the role of *L. acidophilus* and carbohydrate in the etiology of caries was established, the possibility of formulating an activity test, based on these findings, became a research problem for several workers. Their work produced a number of caries activity tests. The most practical of these were those of: (1) Hadley (1933), which consists of counting the number of lactobacilli in the mouth, (2) Wach et al. (1943), a glucose fermentation test which estimates the acid producing power of organisms in the saliva, (3) Snyder (1940), a colorimetric test which depends on a special agar containing an indicator to estimate the amount of acid produced in saliva, and (4) Fosdick et al. (1937), an enamel dissolution test which actually determines the acidity of the saliva by its power to dissolve powdered enamel. These tests were found to give a positive correlation between the test results and the amount of clinical activity as established by fixed standards.
Along with the formulation of activity tests based on the role of the lactobacilli in caries, several control measures have been suggested on this basis. The possible means of caries prevention are: (1) making the tooth resistant to decalcification, (2) preventing the formation of acids on the tooth surface, and (3) neutralizing or buffering the acids before they can attack the enamel. All of these possibilities have been investigated. Although none of the measures suggested have been tested sufficiently for proper evaluation, some present promise as methods of control. These are; (1) immunization against the lactobacilli which has been investigated by Canby and Bernier (1942), and Williams (1944), (2) carbohydrate control, suggested and tested by Jay (1947) and Becks et al. (1946), (3) the use of the antibiotic, penicillin, suggested by Hill (1948), and (4) the use of chemical agents by various methods, such as, the organic mercurials, fluorides, and ammonium carbonate. These measures have in most cases not been carried beyond the experimental stage, but they may in time become highly important in caries control.