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# Association of *Streptococcus mutans* with Human Dental Decay

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The association of *Streptococcus mutans* with human dental decay was investigated by using several types of samples: (i) paraffin-stimulated saliva samples taken from children with from 0 to 15 decayed teeth; (ii) pooled occlusal and approximal plaque taken from children with no decayed or filled teeth, or from children with rampant caries of 10 or more teeth; (iii) plaque removed from single occlusal fissures that were either carious or noncarious. The results showed a significant association between plaque levels of *S. mutans* and caries. The strongest association, $P < 0.0001$, was found when plaque was removed from single occlusal fissures. Seventy-one percent of the carious fissures had *S. mutans* accounting for more than 10% of the viable flora, whereas 70% of the fissures that were caries free had no detectable *S. mutans*. Sixty-five percent of the pooled plaque samples from the children with rampant caries had *S. mutans* accounting for more than 10% of the viable flora, whereas 40% of the pooled samples from children that were caries free had no detectable *S. mutans*. Saliva samples tended to have low levels of *S. mutans* and were equivocal in demonstrating a relationship between *S. mutans* and caries.

*Streptococcus mutans* will cause extensive cavitation of the teeth in several animal species fed high-sucrose diets (21). In these animals, *S. mutans* is the dominant plaque streptococcus (22, 23). *S. mutans* appears to be distributed world-wide in human dental plaque (1) and these human isolates are odontopathic in the animal models (22, 24, 44). An association between *S. mutans* and human decay has been suggested by many studies (3, 9, 14, 16, 17, 19, 24, 28, 31, 34, 36). However, these data are difficult to interpret given the complex dietary, bacterial, and host phenomena involved in human decay. Even studies focusing on the bacteriological aspects of caries are complicated by: (i) the chronic nature of the destructive process, which may take months from its inception to its clinical detection; (ii) the difficulties in diagnosing the incipient carious lesion; (iii) the complex nature and variability of the dental plaque flora (4); and (iv) in particular, the variability of *S. mutans* in the plaque. In recent years, information has accumulated which shows that in humans *S. mutans* often appears to be a minor member of the plaque flora, accounting for less that 15% of the total viable count (3, 27), and that *S. mutans* is found mainly on retentive sites such as carious lesions, pits, occlusal fissures, and approximal surfaces (14, 15, 25, 28). The proportional levels of *S. mutans* in plaque may show a 10,000-fold variation between teeth within the same mouth (43). The last two observations are important because several investigations in the human have either used pooled plaque samples taken from many teeth (5, 17, 35) or have relied extensively upon plaque removed from smooth surfaces (18, 19, 24, 30). This sampling procedure may have greatly underestimated the levels of *S. mutans* by combining plaque from the smooth surfaces which normally do not harbor high levels of *S. mutans* with the quantitatively smaller amounts of plaque taken from the retentive sites which usually are positive for *S. mutans*.

Additional difficulties in associating *S. mutans* with human caries may relate to the methodology employed. Plaque samples are not always plated immediately after collection (17, 31, 34), and there is evidence that the levels of *S. mutans* in plaque samples will decrease upon storage in various transport media (33, 41). *S. mutans* is usually reported as a percentage of the streptococcal flora which grows on mitis salivarius agar containing tellurite (18). This medium, because of the tellurite, may underestimate the counts of *S. mutans* (26, 42). Also, the reporting of the data in this form ignores the majority of the plaque flora

and thus gives a misleading impression of the prominence of *S. mutans* in that flora. These problems have made difficult the interpretation of clinical studies hoping to associate *S. mutans* with human caries.

In the present investigation some of these difficulties were avoided or minimized by sampling plaque from retentive sites and by using culturing techniques and media which appear to optimize the recovery of *S. mutans* (28, 42). The *S. mutans* counts were reported as a percentage of the total colony-forming units (CFU) recovered from the plaque. In addition, saliva samples taken from children whose decayed, missing, and filled teeth (DMFT) scores ranged from 0 to 15 were cultured on a selective medium for *S. mutans* (11). A significant association between percentage levels of *S. mutans* and caries was found (i) in plaques taken from a single occlusal fissure and (ii) in pooled plaques taken from representative occlusal and approximal molar surfaces, present in children who were either caries free, i.e., DMFT = 0, or had rampant caries, i.e., more than 10 decayed teeth. Saliva samples were equivocal in demonstrating a relationship between *S. mutans* and caries.

## MATERIALS AND METHODS

**Subjects.** Three groups of patients were included in this study: pedodontic clinic patients, caries-free patients, and rampant-caries patients. Children who were patients in the pedodontic clinic at The University of Michigan provided either saliva or the occlusal fissure samples. These children were under 10 years of age and their total DMFT scores (a summation of data obtained from primary and permanent teeth) ranged from 0 to 15. The exposure of these patients to water fluoridation varied. The proximity of these subjects to the bacteriology laboratory permitted the culturing of the sample within 1 h after its collection. Thus the data obtained from these samples should exhibit less of the adverse effects that delay in culturing normally introduces.

If *S. mutans* is involved in human caries, then the occurrence and levels of this organism in plaque obtained from caries-free individuals (DMFT = 0) should be considerably lower than in plaque obtained from rampant-caries individuals (decayed teeth greater than 10). The clinic patients did not provide predictable access to either caries-free or rampant-caries children. We were able to find these children in the private practices of two pedodontists. The caries-free children were under 10 years of age and were living in a community whose water supply had been fluoridated for more than 7 years. The rampant-caries patients were children of a similar age living in a community whose water supply was not fluoridated. Plaque was removed and pooled from caries-prone sites such as occlusal and approximal surfaces of molar teeth in these individuals. There was an unavoidable delay of about 24 h in the culturing of these samples because of the distance between the dentists' offices and the laboratory. The samples were added to a reduced transport fluid (RTF) and kept refrigerated until delivered. Another investigation had shown minimal loss of viability of *S. mutans* under these storage conditions (33).

**Detection of caries.** The diagnosis of caries was based on the catch of a dental explorer in a cavitation on the tooth surface and by bite wing radiographs. These criteria would eliminate the white spot lesion from consideration but would include, as caries, surface defects such as developmental grooves. In the study involving single occlusal fissures, the examiner had optimal visibility of the surface so that the most accurate diagnosis would be present in this series. A carious fissure was one in which the fissure had a detectable catch by explorer examination. This tooth was scheduled for restorative treatment and lost from the study. A caries-free fissure was one in which the fissure surface had no detectable catch by explorer examination. The same clinical criteria applied to the examination of the other clinic patients and to the caries-free patients. The rampant-caries patients had open carious lesions which presented no diagnostic problems.

**Collection of sample. (i) Saliva samples; clinic patients.** Clinic patients were given paraffin to chew (39) so as to increase salivation and to remove plague from the tooth surfaces. Several milliliters of saliva were collected and brought immediately to the laboratory for processing. One-milliliter aliquots were removed, added to 9 ml of RFT, and manipulated as described in the microbiological procedures section.

**(ii) Single occlusal fissure sample; clinic patients.** Fissure samples were obtained from molar teeth by means of a pointed wire (27). This wire was fabricated by cutting stainless-steel orthodontic wire into 1-cm pieces and polishing each end to a fine point with a rubber wheel. A separate wire held in the middle with a hemostat was used to remove as much plaque as possible from the fissure of each tooth. Plaque removed from molars containing mesial and distal fossa were treated as separate samples. The wire was immediately placed in 10 ml of RTF and processed within 1 h.

**(iii) Pooled plaque samples; caries-free and rampant-caries patients.** Plaque was removed from approximal and occlusal surfaces of the two most posterior teeth (either deciduous or permanent molars) in each quadrant and pooled so as to give a single sample for each subject. A separate sterile, unwaxed dental floss held in a plastic support (Floss Aid) was used in each quadrant to remove approximal plaque from between the last two molars. In this manner, four floss samples containing plaque removed from eight approximal surfaces were collected and placed in 10 ml of RTF (33, 41). Occlusal fissure samples were obtained by wetting a sterile cotton pellet with RTF and rubbing the occlusal surface with this pellet. A separate pellet was used to sample the two molars in each quadrant. These pellets containing plaque removed from eight occlusal surfaces were placed in

the same RTF as the floss samples. The samples were refrigerated until processed the next day.

**Microbiological procedures.** The pooled plaque, fissure plaque, and saliva samples were processed in a similar manner. The samples were dispersed by sonification for 5 to 10 s at a setting of 6 with the microprobe attachment for the Ultrasonic model W1850 sonifier. This setting and time appeared to give the highest viable count and the highest particle count for plaque bacteria as determined in separate studies in which the sonifier, a Tekmar Tissumizer (Cincinnati, Ohio), and Waring blender were compared. All particles greater than 1 $\mu$m in size were counted with Coulter counter model ZB1. The dispersed samples were serially diluted in RTF, and 0.05-ml aliquots of appropriate dilutions were plated with an Eppendorf pipette over a 3-log dilution range on MM10 sucrose agar (26). The plates were incubated for 5 to 7 days under an atomosphere of 85% $N_2$, 10% $H_2$, and 5% $CO_2$. The MM10 sucrose medium is nonselective and, because of its high sucrose-to-nitrogen ratio, permits the identification of polysaccharide-forming isolates such as *S. mutans*, *S. sanguis*, and *S. salivarius*. The total CFU in each sample was determined and the colonies resembling *S. mutans* and *S. sanguis* were enumerated and expressed as a percentage of the CFU. One or more representative colonies of *S. mutans* from each sample were tested for mannitol fermentation. The salivary samples and some of the fissure samples were plated on a mitis salivarius, 20% sucrose, and 0.2 U/ml bacitracin medium (11) minus the addition of potassium tellurite (42). This medium is highly selective for *S. mutans* and permits its detection when present in low numbers. The *S. mutans* and *S. sanguis* counts were done by one individual and verified by a second.

**Statistical analysis.** The statistical analyses were done by computer using the Midas program of the Michigan Terminal System. Both parametric and nonparametric, i.e., chi-square, Kruskall-Wallis one-way analysis of variance, and median tests, were used when appropriate (38).

## RESULTS

**Saliva samples.** Salivary cultures were taken to determine whether it would be possible to demonstrate that salivary levels of *S. mutans* are a function of the number of carious teeth in the mouth. The levels of *S. mutans* in saliva are usually less than 1% of the CFU (6) and thus difficult to detect on nonselective media. Recently a selective medium for *S. mutans*, the mitis-sucrose-bacitracin agar, has been described (11) which permits its quantitative recovery from a sample when present in low numbers. This medium and MM10 sucrose agar were used to determine the levels of *S. mutans* in the saliva of patients seen at their first visit to the pedodontic clinic. The number of decayed teeth in these patients ranged from 0 to 15. Fifteen patients had no detectable *S. mutans*, i.e., less than $10^4$ organisms/ml of saliva.

The patients were stratified into eight groups according to the number of decayed teeth, and the total count, percentage of *S. mutans*, and percentage of *S. sanguis* for each group were determined (Table 1). The *S. mutans* levels were low in the eight caries-free individuals accounting for 0.16% of the salivary flora. The presence of one to four carious teeth did not increase the proportions of *S. mutans* in the saliva. In the groups with five to 10 carious teeth, *S. mutans* averaged 1.12 to 2.25% of the total flora (Table 1). However, in the six individuals that had 11 or more carious teeth, the percentages of *S. mutans* dropped to low levels. *S. sanguis* accounted for 1 to 4.1% of the flora and was proportionately higher than *S. mutans* in all groups with the exception of the 12 individuals with seven to eight carious teeth.

The data was analyzed by the Kruskall-Wallis ranking test and the median test. In the Kruskall-Wallis test the percentages of *S. mutans* for each saliva sample were ranked from low to high. Then, for each group described by the number of decayed teeth, the average rank assigned to the corresponding *S. mutans* percentages was determined. Table 2 shows that there was no significant increase in the average rank of *S. mutans* as the number of decayed teeth increased. The relationship between increasing numbers of carious teeth and the median percentage of *S. mutans* in the saliva, i.e., 0.2%, was not significant (Table 2). However, if the subjects were stratified according to the number of decayed and filled teeth, a significant association was found with the Kruskall-Wallis test (Table 3). This was due mainly to the large number of subjects with seven to 10 decayed or filled teeth who had levels of *S. mutans* greater than 0.2% of

TABLE 1. *Relationship between number of decayed teeth and salivary levels of S. mutans and S. sanguis*

| No. of decayed teeth | No. of subjects | Total count[a] ($\times 10^6$/ml) | Percentage of *S. mutans*[b] | Percentage of *S. sanguis*[a] |
|---|---|---|---|---|
| 0 | 8 | 88.2 ± 13.6[c] | 0.16 ± 0.04 | 1.1 ± 0.4 |
| 1-2 | 7 | 166.9 ± 59.4 | 0.35 ± 0.16 | 4.1 ± 1.1 |
| 3-4 | 11 | 184.1 ± 49.6 | 0.13 ± 0.06 | 1.3 ± 0.5 |
| 5-6 | 8 | 144.6 ± 53.7 | 1.33 ± 1.00 | 1.8 ± 1.19 |
| 7-8 | 12 | 101.3 ± 11.9 | 2.25 ± 0.85 | 1.0 ± 0.3 |
| 9-10 | 8 | 109.1 ± 24.2 | 1.12 ± 0.46 | 2.1 ± 0.7 |
| 11-12 | 4 | 169.5 ± 116.1 | 0.29 ± 0.27 | 1.0 ± 0.4 |
| >12 | 2 | 172.5 ± 57.5 | 0.07 ± 0.06 | 2.4 ± 0.8 |

[a] Total counts and *S. sanguis* count obtained from MM10 sucrose agar after 4 to 7 days anaerobic incubation.

[b] *S. mutans* count taken from mitis salivarius, bacitracin, and 20% sucrose agar after 4 to 7 days anaerobic incubation.

[c] Average ± standard error of the mean.

TABLE 2. *Relationship between the median percentage of S. mutans in saliva and the total number of decayed teeth*

| No. of decayed teeth | No. of subjects in each group | Average rank of each group[a] | Median % of *S. mutans* (0.2%)[b] | |
|---|---|---|---|---|
| | | | L | G |
| 0 | 8 | 26.7 | 4 | 4 |
| 1-2 | 7 | 28.8 | 4 | 3 |
| 3-4 | 11 | 21.3 | 8 | 3 |
| 5-6 | 8 | 32.3 | 3 | 5 |
| 7-8 | 12 | 40.5 | 4 | 8 |
| 9-10 | 8 | 37.6 | 2 | 6 |
| 11-12 | 4 | 21.4 | 3 | 1 |
| >12 | 2 | 24.5 | 2 | 0 |

[a] Kruksall Wallis test, 10.1; df, 7; *P*, 0.18.

[b] L, Number of subjects in each group whose percentage of *S. mutans* was less than the median value; G, number of subjects in each group whose percentage of *S. mutans* was greater than the median value. Median test, 9.1; df, 7; *P*, 0.25.

the flora. This significance came despite the low percentages of *S. mutans* in the 12 individuals with 11 or more involved teeth.

**Pooled fissure and approximal plaque.** Plaque samples were collected from two populations of children who represented clinical extremes as regards to dental caries, i.e., the caries-free child and the rampant-caries child. Pooled plaque samples were collected from retentive sites, i.e., occlusal and approximal surfaces of molar teeth, in 27 caries-free children and in 43 children with 10 or more carious teeth. The subjects in each population were stratified according to their plaque levels of *S. mutans*, i.e., *S. mutans:* (i) was not detected, being less than $0.1 \times 10^4$ organisms/sample; (ii) accounted for less than 1% of the CFU; (iii) accounted for 1 to 10% of the CFU; (iv) accounted for greater than 10% of the CFU. A frequency distribution of the caries-free and rampant-caries children in these cells showed, by chi-square analysis, a highly significant association between increasing percentage of *S. mutans* and rampant caries, i.e., $P = 0.001$ (Table 4). Sixty-three percent of the plaques taken from caries-free subjects had low proportions, i.e., <1%, of *S. mutans*, whereas only 16% of the plaques taken from rampant-caries subjects exhibited similar low percentages of *S. mutans*.

The total number of CFU recovered from the plaques was comparable in the caries-free and the rampant-caries subjects (Table 5), suggesting no difference between the subjects as regards to plaque mass removed from the sites sampled. *S. mutans* was a dominant organism, comprising about 27 to 32% of the flora in 65% of the rampant-caries individuals and in 30% of the caries-free individuals. These high levels of *S. mutans* were associated with low levels of *S. sanguis*, so that the ratio of *S. mutans* to *S. sanguis* (the MS ratio) was greater than unity (28) in these subjects. Conversely, where the *S. mutans* levels were low, the *S. sanguis* levels were higher giving rise to ratios less than 0.2 (Table 5).

**Single occlusal fissure sites.** Plaque was removed from 253 occlusal fissures present in the teeth of 80 patients seen in the pedodontic clinic. One hundred and thirty fissures were judged to be carious and 123 fissures to be caries free by the presence or absence of surface cavitation. More organisms were recovered from the carious fissures than from the caries-free fissures, but the difference was not significant (Table 6). *S. mutans* was significantly higher in the carious sites and *S. sanguis* was significantly lower (Table 6). The MS ratio was 2.56 in the plaque taken from carious fissures and 0.55 in the plaque from caries-free sites. These data would indicate that bacterial numbers were not as important as bacterial composition in the determination of caries activity.

The fissures were placed into one of four groups according to their plaque levels of *S. mutans* as described for the pooled plaque samples previously. When the Kruskall-Wallis and median tests were applied to this distribu-

TABLE 3. *Relationship between the median percentage of S. mutans in saliva and the total number of decayed and filled teeth*

| No. of decayed and filled teeth | No. of subjects in each group | Average rank of each group[a] | Median % of *S. mutans* (0.2%)[b] | |
|---|---|---|---|---|
| | | | L | G |
| 0 | 7 | 25.1 | 4 | 3 |
| 1-2 | 2 | 24.0 | 3 | 2 |
| 3-4 | 5 | 21.0 | 2 | 0 |
| 5-6 | 10 | 26.3 | 6 | 4 |
| 7-8 | 10 | 39.4 | 3 | 7 |
| 9-10 | 14 | 42.8 | 3 | 11 |
| 11-12 | 8 | 22.6 | 5 | 3 |
| >12 | 4 | 16.3 | 4 | 0 |

[a] Kruskall-Wallis test, 14.7; df, 7; *P*, 0.04.

[b] L, Number of subjects in each group whose percentage of *S. mutans* was less than the median value; G, number of subjects in each group whose percentage of *S. mutans* was greater than the median value. Median test, 13.3; df, 7; *P*, 0.06.

tion, the association between increasing percentages of *S. mutans* and a carious fissure was significant, i.e., $P < 0.0001$ (Table 7). Seventy-one precent of the fissures whose plaque had greater than 10% *S. mutans* were carious, whereas 70% of the fissures that were caries free had no detectable *S. mutans*. However, it was possible to have a carious fissure without detectable *S. mutans*, as well as to find high levels of *S. mutans* in fissures that were not considered carious (Tables 7 and 8).

The actual numbers of bacteria removed from the fissures varied from $0.6 \times 10^4$ to $1,870 \times 10^4$. The average viable recoveries in each of the eight categories ranged from 95 to $324 \times 10^4$ (Table 8). The percentages of *S. mutans* and *S. sanguis* and the MS ratios in each of the eight categories were calculated (Table 8). An inverse relationship between *S. mutans* and *S. sanguis* was present giving rise to high MS ratios in most carious fissures and lower values in most caries-free fissures. *S. mutans* was a dominant member of the flora, averaging 32 to 35% of the isolates in half of the carious fissures and in one-fifth of the noncarious fissures. *S. sanguis* averaged from 5.2 to 23.2% of the isolates, which were higher proportions than those seen previously in the pooled plaque samples (Table 5).

TABLE 4. *Frequency distribution of S. mutans as a percentage of the CFU isolated from pooled plaque samples taken from either caries-free or rampant-caries children*[a]

| Children in which *S. mutans*: | No. of children | |
|---|---|---|
| | Caries free | Rampant caries |
| Was not detected | 11 (6.6)[b] | 6 (10.4) |
| <1% of the CFU[c] | 6 (2.7) | 1 ( 4.3) |
| 1 to 10% of the CFU | 2 (3.8) | 8 ( 6.1) |
| >10% of the CFU | 8 (13.9) | 28 (22.2) |

[a] $\chi^2 = 16.68$; df = 3; $P = 0.001$.
[b] Expected values are in parentheses.
[c] CFU on MM10 sucrose agar.

## DISCUSSION

A relationship between *S. mutans* and human dental decay was shown at three levels of observation. The strongest association was found when plaque was removed from single occlusal fissures and cultured immediately. The carious fissures, many of which appeared clinically to be incipient enamel lesions, had significantly higher proportions of *S. mutans* in their overlying plaque than did most caries-free fissures. There was a corresponding significant decrease in the proportions of *S. sanguis* in the plaques taken from carious fissures when compared to the plaques taken from caries-free fissures (Table 8). A similar pattern was observed in pooled plaque samples when caries-free individuals were compared to rampant-caries individuals. The rampant-caries children had appreciably more *S. mutans* in their plaques

TABLE 5. *Percentage of S. mutans and S. sanguis in pooled plaque samples taken from either caries-free or rampant-caries children*

| *S. mutans* | Total CFU[a] × $10^4$ | % *S. mutans* | % *S. sanguis* | M/S[d] |
|---|---|---|---|---|
| Caries free | | | | |
| not detected | 1,071 | 0.0 | 5.4 ± 8.0[c] | <0.1 |
| n = 11 | (86–3,425)[b] | | | |
| <1% of CFU | 3,901 | 0.4 ± 0.3 | 2.8 ± 4.3 | 0.14 |
| n = 6 | (910–10,950) | | | |
| 1 to 10% of CFU | 1,138 | 4.8 ± 2.8 | 0.8 ± 0.7 | 6.0 |
| n = 2 | (1,085–1,190) | | | |
| >10% of CFU | 3,060 | 32.9 ± 22.9 | 2.8 ± 6.5 | 11.78 |
| n = 8 | (112–11,700) | | | |
| Rampant caries | | | | |
| Not detected | 1,410 | 0.0 | 11.3 ± 14.1 | <0.1 |
| n = 6 | (57–6,800) | | | |
| <1% of CFU | 214 | 0.9 | 8.9 | 0.1 |
| n = 1 | | | | |
| 1 to 10% of CFU | 1,472 | 3.8 ± 1.8 | 6.1 ± 10.1 | 0.62 |
| n = 8 | (251–4,950) | | | |
| >10% of CFU | 2,026 | 26.8 ± 16 | 4.5 ± 7.1 | 5.95 |
| n = 28 | (139–11,275) | | | |

[a] Total CFU on MM10 sucrose plates after 5 to 7 days anaerobic incubation.
[b] Range of CFU.
[c] Average ± standard deviation.
[d] M/S is % *S. mutans* to % *S. sanguis* ratio.

than did the caries-free children. The differences would have been even more striking except for the presence of eight caries-free children with unusually high levels of *S. mutans* in their plaques (Table 5). *S. mutans* averaged 18% of the plaque flora in the rampant-caries children. This value is higher than that found previously in similarly obtained samples from an adolescent population (27) and is suggestive of a *S. mutans* infection in these individuals. The MS ratio in the pooled samples was similar to that observed in the single site samples. The pooled plaque samples exhibited an exaggerated standard deviation relative to the other samples. This could reflect both the 24-h delay in culturing and/or the fact that, at least in the rampant-caries children, plaque could have been included from both carious and caries-free sites. The pooled plaque samples were adequate to demonstrate the differences in *S. mutans* levels in the two populations studied. This suggested that similar samples obtained from retentive sites of molar teeth in each quadrant could be used on a screening basis to detect high levels of *S. mutans* in other subjects. This would be a more practical way of gauging whether an individual's teeth are colonized by *S. mutans* than to individually culture each tooth surface.

The weakest association between *S. mutans* and decay occurred when paraffin-stimulated saliva samples were tested. This would be expected because the salivary flora is derived mainly from the tongue and soft tissues (10) and the contribution of the plaque, including plaque

TABLE 6. *Percentage of S. mutans and S. sanguis in plaque samples taken from a single fissure diagnosed as caries free or carious*

| Determinants | Fissures | | Significance[a] |
|---|---|---|---|
| | Caries free | Carious | |
| Total count ($\times 10^4$) | 105 ± 17.5[b] | 148.3 ± 21.3 | $P = 0.12$ |
| % *S. mutans* | 7.3 ± 1.3 | 18.7 ± 1.9 | $P < 0.0001$ |
| % *S. sanguis* | 18.6 ± 1.6 | 10.3 ± 1.1 | $P < 0.0001$ |

[a] n = 253. Student *t* test.
[b] Average ± standard error of the mean.

TABLE 7. *Frequency distribution of S. mutans as a percentage of the CFU isolated from single occlusal fissures which were either clinically caries free or carious*[a]

| Sites in which *S. mutans*: | No. of fissures | |
|---|---|---|
| | Caries free | Carious |
| Was not detected | 70 | 30 |
| <1% of the CFU[b] | 10 | 3 |
| 1 to 10% of the CFU | 17 | 32 |
| >10% of the CFU | 26 | 65 |

[a] Kruskall-Wallis, 30:5; $P < 0.0001$. Median test, 40.7; $P < 0.0001$.
[b] CFU on MM10 sucrose agar after 5 to 7 days of anaerobic incubation.

TABLE 8. *Percentage of S. mutans and S. sanguis in plaque samples taken from a single occlusal fissure diagnosed as caries free or carious*

| *S. Mutans* | Total count ($\times 10^4$) | % *S. mutans* | % *S. sanguis* | M/S (%)[c] |
|---|---|---|---|---|
| Caries free | | | | |
| Not detected | 120 + 25[a] | 0.0 | 23.2 ± 2.4 | 0.1 |
| (n = 70) | (2.0 - 1000)[b] | | | |
| <1% of CFU | 324 ± 146 | 0.4 ± 0.1 | 17.4 ± 6.1 | 0.03 |
| (n = 10) | (2.6 - 1250) | | | |
| 1-10% of CFU | 95 ± 43 | 3.4 ± 0.4 | 15.6 ± 3.4 | 0.22 |
| (n = 17) | (1.3 - 600) | | | |
| >10% of CFU | 126 ± 38 | 31 ± 2.9 | 8.5 ± 1.8 | 3.75 |
| (n = 26) | (0.6 - 600) | | | |
| Carious | | | | |
| Not detected | 145 ± 43 | 0 | 16.8 ± 2.6 | <0.1 |
| (n = 30) | (2.1 - 900) | | | |
| <1% of CFU | 249 ± 140 | 0.5 ± 0.1 | 11.5 ± 2.1 | 0.04 |
| (n = 3) | (126 - 500) | | | |
| 1 to 10% of CFU | 155 ± 48 | 4.3 ± 0.4 | 14.1 ± 2.8 | 0.30 |
| (n = 32) | (2.0 - 900) | | | |
| >10% of CFU | 188 ± 37 | 35.0 ± 2.4 | 5.2 ± 0.7 | 6.73 |
| (n = 65) | (4.0 - 1,870) | | | |

[a] Average ± standard error of the mean.
[b] Range of counts.
[c] M/S is % *S. mutans* to % *S. sanguis* ratio.

over a carious lesion, to this flora would be minimal. This relatively insensitive saliva culture showed that *S. mutans* increased from 0.16% of the cultivable flora in the absence of caries, to 0.35% in the presence of one to two lesions, to a high of 2.25% in the presence of seven to eight lesions and then decreased at higher numbers of decayed teeth. No significance between the number of decayed teeth and increasing proportions of *S. mutans* in the saliva could be shown by nonparametric analysis. However, when the comparison was between the number of decayed and filled teeth and increasing proportions of *S. mutans*, significance was observed (Table 3). This would indicate that *S. mutans* remains on surfaces after they are restored with dental alloys confirming the observation of Keene et al. (20).

These results leave little doubt that *S. mutans* is somehow involved in human decay at the time when cavitation is present. However, at each level of sampling, certain individuals or teeth had high levels of *S. mutans* without clinically detectable cavitation. This can be attributed to several complex interrelated phenomena such as sucrose content of the diet, eating habits, brushing habits, fluoride content of the tooth and plaque, possible immune mechanisms in the saliva, genetic factors, and inherent characteristics of *S. mutans*. It should be noted that the caries-free children with the elevated levels of *S. mutans* resided in a fluoridated community. We shall confine our discussion to those aspects which relate specifically to *S. mutans*. First, *S. mutans* may not be a pathogen in each instance. At least three possible species on the basis of genetic analysis (2) are currently classified as *S. mutans*. It is possible that one or more of these genetic groupings is nonpathogenic in humans. Second, *S. mutans* may be but one member of a mixed infection which is responsible for cavitation. If the other organisms are absent, then cavitation may not occur. Third, the ability of *S. mutans* to cause cavitation may be neutralized and/or modified by other members of the plaque flora. In gnotobiotic animals, when a *Veillonella* species was combined with *S. mutans*, the amount of cavitation decreased (29). Several members of the human plaque flora produce glucan hydrolases (40) which may in vivo degrade the extracellular glucans formed by *S. mutans*. In animal experiments, the enzymatic removal of these glucans decreases the amount of decay (8, 13). Fourth, the presence of *S. mutans* may indicate an early stage of infection that cannot be detected by clinical examination. The initial carious lesion is a subsurface demineralization (12) that precedes cavitation by an unknown period of time. It is at this stage of the lesion, before cavitation occurs, that dental therapy should be addressed, because this demineralization may be reversed with various calcifying solutions. Hence, the presence of *S. mutans* on a surface might be the indication for prompt chemotherapy of the surface. In the present instance, the caries-free surfaces colonized with *S. mutans* are being monitored at 6-month intervals to determine their subsequent clinical fate. Recent studies indicate that teeth colonized by *S. mutans* are more likely to develop caries in the succeeding year than are teeth which have no detectable levels of *S. mutans* (16, 19).

The data show that rampant-caries individuals and caries-active fissures were found that had no detectable levels of *S. mutans*. Also, the levels of *S. mutans* in saliva decreased in the groups with 10 or more carious surfaces. These observations imply, among other possibilities, that in certain substances organisms other than *S. mutans* may be responsible for the caries observed. In animal models, several species are capable of causing cavitation, particularly of fissure surfaces (7, 22, 32). Some of the cavitation found in the present fissure study may reflect catches due to developmental defects which were misdiagnosed as caries. This explanation would not apply to the rampant caries and saliva studies where 10 or more decayed teeth were present. Another partial explanation for the absence of *S. mutans* could be the bacterial succession that occurs in the carious lesion. As the decay progresses into the dentine, the microenvironment appears to select for aciduric organisms such as *Lactobacilli* (26, 37). In the dentinal lesion *S. mutans* is recovered from about half the samples (26; W. J. Armstrong, M.S. thesis, Univ. of Michigan, Ann Arbor, 1974). Thus, in the individual or tooth with dentinal involvement, the plaque and saliva may have reduced levels of *S. mutans*.

In a chronic disease such as dental decay, difficulties are encountered in attributing overt pathogenicity to only one member of a complex flora. This is even more so when the earliest clinical manifestation of the disease, i.e., cavitation, is already a terminal event. Until more precise diagnostic criteria become available for the early lesion, the presence of *S. mutans* in high numbers, i.e., >1% of the CFU, on a surface may be the foremost indicator of the future health of that surface. However, this requires an assumption that *S. mutans* is a human dental pathogen, which cannot be observed from cross-sectional data of the type

presented in this investigation. This dilemma may be resolved by more precise longitudinal studies which show that *S. mutans* colonization precedes caries and by elimination studies which show that surfaces rid of *S. mutans* have lower caries experience than surfaces which retain *S. mutans*.

The fact that *S. mutans* averages only 19% of the cultivable flora (Table 6) even in the carious fissures indicates that the other members of flora may be important modifiers of *S. mutans* behavior in the plaque. The precise identity of these organisms is not known, but preliminary studies indicate that they are mainly other *Streptococcus* species, *Antinomyces* species, and *Veillonella* species (26).

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