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Longitudinal Investigation of the Role of *Streptococcus mutans* in Human Fissure Decay

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A prospective study was initiated in order to detect changes in the levels and proportions of *Streptococcus mutans*, *S. sanguis*, and lactobacilli before and at the time of caries development on occlusal fissures. The bacteriological analysis was performed on 195 teeth that received four examinations at approximately 6-month intervals. The data obtained from 42 carious fissures and 153 caries-free fissures strongly indicated an etiological role for *S. mutans* in most of the diagnosed fissure lesions. This was demonstrated by the longitudinal analysis, which showed the proportions of *S. mutans* to increase significantly at the time of caries diagnosis, and by cross-sectional comparisons, which showed that the proportions of *S. mutans* in the carious fissures were significantly higher than in caries-free fissures. Three subjects who had a low caries experience developed five new carious lesions. Lactobacilli were prominent members of the caries-associated flora in these subjects, greatly outnumbering *S. mutans*. The levels and proportions of *S. sanguis* tended to be higher in the caries-free fissures. Although the results are striking in that they implicate *S. mutans* in fissure decay, they show that clinical decay can occur in a few instances in the absence of detectable *S. mutans*, as was observed in the fissures high in lactobacilli.

Most human teeth that are diagnosed as decayed have significantly elevated levels and proportions of *Streptococcus mutans* compared with teeth diagnosed as caries-free (4, 5, 8, 12, 19, 23, 32, 34). On the same tooth, plaque which is removed from a carious site differs significantly in its *S. mutans* composition and in its rate and amount of sucrose utilization from plaque removed at the same time from a non-carious site (28). Human isolates of *S. mutans* are overt dental pathogens in animals ingesting sucrose diets (16, 25, 39). Although these findings indicate a prominent role for *S. mutans* in human dental decay (16, 23), there has been minimal application of this knowledge in clinical dentistry, other than in rare instances where the salivary or plaque levels of *S. mutans* were used to identify caries-susceptible patients (18, 22, 38) and to monitor treatment efficacy (18, 22, 38). This in part reflects the inadequacy of cross-sectional, association-type studies to indicate whether the high proportions of *S. mutans* in the carious lesion are the cause or the result of the dental decay. Longitudinal studies presumably would demonstrate whether the increase in *S. mutans* coincided with caries development. A prospective clinical study to detect changes in the tooth surface flora before and at the time of caries development would be expensive, since it would have to be large enough to accommodate the uncertainties of patient cooperation and the likelihood of any given tooth surface becoming carious during the period of observation. The findings would have to be interpreted against the variability related to diet, fluoride exposure, use of antimicrobial medication, oral hygiene habits, salivary composition and flow, microbial interactions in the plaque, and host immunological experience, among other factors. These considerations indicate that the idealized study will not be performed and that practical longitudinal investigations will have certain limitations inherent in their design.

Most longitudinal investigations support a role for *S. mutans* in the development of decay (6, 13, 14, 19, 33, 35, 38), with a few exceptions (11, 26). In some studies, few of the teeth sampled eventually became decayed (11, 13, 19, 38); in others, the bacteria were studied in saliva (6) or pooled plaque (19, 35, 38) and not on the tooth surfaces which subsequently became carious. In most instances (6, 13, 14, 19, 26, 33, 35, 38), primarily the streptococcal segment of the oral flora was examined, thereby omitting the contribution of the other plaque organisms to the decay process. Preliminary reports from the only longitudinal study to date, in which representative members of the total plaque flora are

being identified, are equivocal in demonstrating a prominent role for *S. mutans* in the development of carious lesions on the distal approximal surface of first premolars (2, 11). However, in this study, the method of plaque sampling was such that the plaque associated with the caries-prone site could have been diluted with supragingival plaque from the gingival margin.

The present investigation overcame some of the above-described difficulties by sampling approximately 200 teeth repeatedly on the same occlusal surfaces in which the decay was being monitored.

MATERIALS AND METHODS

Subjects. All patients were seen in the pedodontic department of the University of Michigan School of Dentistry. All carious teeth were restored before the patient entered the study. Currently, 52 patients have remained in the study long enough to have received four examinations at approximately 6-month intervals. This study population was comprised of 22 females and 30 males who, at the onset of the study, ranged from 5 to 12 years of age. Bacteriological analyses were performed on about four teeth per patient and included both primary and permanent molars.

Collection of plaque. Occlusal fissure plaque samples were obtained from molar teeth by means of 26-gauge Yale hypodermic needles (Becton, Dickinson and Co., Rutherford, N.J.). The needle was held in the middle with a hemostat, and the end with the plastic hub was broken off. The needle tip was firmly scratched along the entire fissure length and dropped into a 10-ml tube of reduced transport fluid (RTF) (24). Plaque removed from molars containing mesial and distal fossa were treated as separate samples. The RTF tube was brought to the laboratory, and the plaque samples were processed within 20 to 30 min after collection. After collection of the plaque sample, the fissures were examined for the presence or absence of decay.

Detection of decay. All clinical examinations were performed by a single individual using a standard dental explorer (no. 3 Wesco explorer). The diagnosis of caries was based on the catch of the explorer in a cavitation in the occlusal fissure. This criterion would eliminate the white spot lesion from consideration, but might include as caries any surface defects which enlarged during the period of observation. Bite wing radiographs were taken at each visit; in no instance did they exhibit evidence of decay, indicating that what was recorded as decay represented very early lesions. The ethical guidelines of the study required that all surfaces diagnosed as decayed be promptly restored, which precluded observing the lesion to determine the rate, if any, at which it progressed. However, this experimental design brought the patient back into the pedodontic clinic, where the diagnosis of caries had to be confirmed by a staff pedodontist before dental restoration was placed. For 42 of 43 teeth judged decayed by the study pedodontist, the diagnosis was confirmed by the second pedodontist. The caries-free teeth were caries-free for four consecutive examinations. However, to ensure that the last caries-free data point entered would not later be found to be an immediately precarious data point, only the data for the first three examinations are presented. Thus, the 153 caries-free teeth reported upon were known to be caries-free for 6 months after what is listed as the penultimate diagnosis, or zero-time value in Tables 2 through 8.

Bacteriological procedures. The fissure samples were dispersed by sonication for 5 s at a setting of 6 with the microprobe attachment for the Ultrasonic model W1850 Sonifier (Heat Systems-Ultrasonics Inc., Plainview, N.Y.). The dispersed samples were serially diluted in RTF, and appropriate dilutions were automatically plated with a spiral plater (Spiral System, Inc., Cincinnati, Ohio) over a 2- to 4-log dilution range on MM10 sucrose agar (24), MSB agar (10), and LBS agar (30). The plates were incubated for 5 to 7 days under an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. Colonies appeared on a spiral trace in which the number of colonies decreased from the center outward. To calculate the number of colony-forming units (CFU) in the plaque sample, the agar surface was ruled into 45° pie-shaped sectors by means of a precision-made stainless-steel "cookie cutter." The sectors were then partitioned into smaller segments (Fig. 1). The number of CFU in each segment or sector was multiplied by the reciprocal of the volume of the inoculum delivered to each segment or sector and by the dilution factor, in order to calculate the number of CFU present in the inocula. The dilution factors were set so that plaque samples containing 330 to 10^8 CFU could be conveniently counted. The MM10 sucrose medium was used for the total count and for the *S. mutans* and *S. sanguis* counts. All *S. mutans* and *S. sanguis* counts were done by one individual and veri-

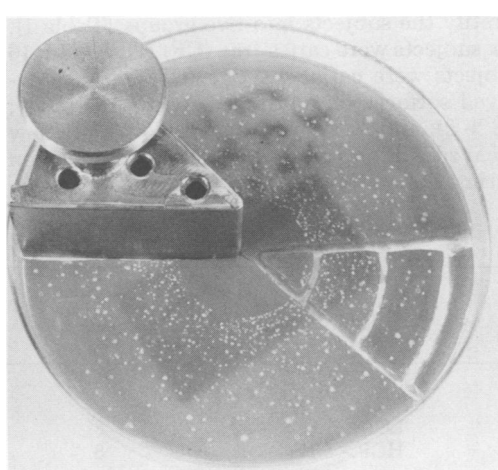

FIG. 1. *Agar plate inoculated on spiral plater. Note how the colonies on the spiral trace decrease at the periphery. A precision-made "cookie cutter" is used to cut sectors in the agar, which permits enumeration of the CFU in a known area that has received a known volume of the inoculum.*

fied by a second. The *S. mutans* counts on the MSB agar tended to be lower than those obtained on the MM10 sucrose agar (22) and were only used when the *S. mutans* colonies on the MM10 sucrose agar plate were so few that *S. mutans* could not be reliably counted. The LBS medium was used for the lactobacilli counts. The proportions of *S. mutans*, *S. sanguis*, and lactobacilli were obtained by dividing the counts of these organisms by the total count on the same or the corresponding MM10 sucrose plate. However, in order to avoid undue distortion of this percent-normalized data, no proportions were calculated if fewer than 20 CFU were present on this plate. In computing the *S. mutans*/*S. sanguis* ratio (21), a value of 10 was used when *S. mutans* was present and *S. sanguis* was absent. Because of the nonlinearity of this ratio, nonparametric statistical tests were used to evaluate the differences between groups.

Statistical analysis. The statistical analyses were performed by computer, using the MIDAS program of the Michigan terminal system. The values obtained at the time of the third consecutive caries-free or caries diagnosis were compared with the corresponding values obtained at the prior examinations by both a parametric paired *t* test and the nonparametric Wilcoxon rank *t* test. Differences between groups were compared by the Student *t* test, Mann-Whitney *U* test, and one-way analysis of variance (ANOVA), using the Scheffe test to compare differences between groups.

RESULTS

Clinical groups. The caries experience of the subjects varied from 0 to 40 decayed-filled tooth surfaces (DFS). The subject's past caries experience as measured by the DFS score plus caries activity during the study period was used to stratify the subjects into five groups (Table 1). Six subjects were caries-free (CF) (DFS = 0); 16 subjects were caries-inactive (CI) (no new decayed surfaces on any tooth during the study; FS > 0); and 30 subjects had one or more new decayed surfaces somewhere in their dentition during the study and were therefore caries-active (CA) (DFS > 0). Within the CI and CA groups, subgroups were defined by using a caries score of 5 as the partition and sorting the subjects into low- and high-caries groups. The CI group was divided into a low (L) and a high (H) CI group that were matched by age but differed significantly ($P = 0.01$) in total caries experience (4.2 DFS in the LCI group versus 16.9 DFS in the HCI group (Table 1). The CA group had only three subjects in the LCA group and 27 in the HCA group. Both groups had the same mean age, but differed significantly ($P < 0.05$) in their total caries experience (4.8 DFS in the LCA group versus 21.6 DFS in the HCA group). The difference in caries experience between the low and high CI and CA groups was found in both the primary and permanent teeth (Table 1). The CF group differed from the other groups in being significantly younger at entry into the study. These five groups, CF, LCI, HCI, LCA, and HCA, served as the basis for analyzing the bacteriological data.

Plaque microbial analysis. The bacteriological status of the fissures in the various groups at the time of the last diagnosis was determined. The total viable counts on the carious fissures in the LCA and HCA groups were higher than on the caries-free fissures in the same groups, but these differences were not significant (Table 2). However, the counts on the caries-free fissures in the HCA group were significantly lower than the corresponding counts in the CF and HCI groups. When the *S. mutans* levels in all the groups were compared by the two-sample *t* test and the Mann-Whitney *U* test, the differences between the HCA and HCI and the LCA and LCI were generally significant ($P < 0.05$). The levels of lactobacilli were significantly higher in the carious teeth in LCA subjects than in the fissures of the other groups, except for the CF group (Table 2).

TABLE 1. *Age and caries experience of patients in longitudinal caries study*

Caries status	No. of patients	Age at entry	DFS		
			Primary teeth	Permanent teeth	Total
CF	6	5.2[a]	0	0	0
CI					
LCI ≤ 5 FS	8	7.6	2.3[b]	1.9[b]	4.2[b]
HCI > 5 FS	8	8.7	11.2	5.7	16.9
CA					
LCA ≤ 5 DFS	3	8.5	2.6[c]	2.2[c]	4.8[c]
HCA > 5 DFS	27	8.5	14.2	7.4	21.6

[a] Significantly different from all other groups: ANOVA, using the Scheffe test.
[b] LCI significantly less than HCI; ANOVA, using the Scheffe test.
[c] LCA significantly less than HCA; ANOVA, using the Scheffe test.

The counts for the various species were normalized as a percentage of the total viable count for each plaque sample, and the mean and median values are given in Tables 3 through 7. *S. mutans* accounted for 24.6% of the cultivable flora in the carious fissures of the HCA subjects. This value was significantly higher than the proportions of *S. mutans* found in the fissures in the other groups (Table 3). The median value of 18.6% was also significantly different from the median values observed in the other groups. *S. mutans* was present in very low proportions in the carious fissures of the LCA subjects and was not detected in the six caries-free fissures in these subjects. The caries-free fissures in the HCI and HCA subjects had about 8% of their flora as *S. mutans*, a value which was significantly higher than the 2% found in the caries-free fissures of the CF and LCI subjects (ANOVA, $P = 0.06$; Scheffe test, $P < 0.05$). The proportions of *S. sanguis* ranged from 4.6 to 20.6%, with the value in the LCI fissures being significantly higher than values obtained for the other fissures (Table 3). Lactobacilli either were not detected or were present in low proportions in the caries-free fissures and in the carious fissures of the HCA group (Table 3). However, in the carious fissures of the LCA group, lactobacilli accounted for 4% of the flora, a proportion which was significantly higher than that found in the other groups (Table 3). The *S. mutans/S. sanguis* ratio was significantly higher in the carious fissures of the HCA group than in the other fissures (Table 3). The mean value of this ratio was relatively high in the caries-free fissures of the HCI and HCA groups, possibly reflecting the cariogenic challenge present in these subjects.

TABLE 2. *CFU of fissures at time of caries diagnosis or penultimate caries-free diagnosis*

Caries status of tooth	No. of teeth	Mean CFU × 10^4/Fissure			
		Total	*S. mutans*	*S. sanguis*	Lactobacilli
Carious					
LCA	5	150	0.09	6.3	7.9[a]
HCA	37	116	10.9	32.4	0.8
Caries-free					
CF	24	170	0.1	6.6	3.1
LCI	31	115	2.3	14.5	ND[b]
HCI	24	179	8.3	9.9	0.16
LCA	6	41	ND	2.1	ND
HCA	68	46[c]	9.1	4.4	0.12

[a] Significantly higher than all values in column except for CF group; $P < 0.05$ (ANOVA, using Scheffe test).
[b] ND, Not detected.
[c] Significantly lower than the values in the CF and HCI groups; $P < 0.05$ (ANOVA, using Scheffe test).

The bacteriological values obtained at the time of the caries diagnosis or of the penultimate caries-free diagnosis (zero time in Tables 4 through 8) were compared with the corresponding values obtained 6 and 12 months before this diagnosis. The percentages for the zero-time value were slightly different from those shown in Table 3 because we had to omit an occasional zero-time value from the paired statistical tests when a missing value occurred at either the −6- or −12-month visit.

The mean and median percentages of *S. mutans* in plaque removed from carious fissures in the HCA subjects increased significantly at the time of caries diagnosis compared with the proportions seen 6 and 12 months before this diagnosis (Table 4). No other fissures showed significant changes in the proportions of *S. mutans* during this time span. *S. mutans* was a minor

TABLE 3. *Percent-normalized values of fissures at time of caries diagnosis or penultimate caries-free diagnosis*

Caries status of tooth	Percent-normalized value							
	S. mutans		*S. sanguis*		Lactobacilli		*S. mutans/S. sanguis*	
	Mean	Median	Mean	Median	Mean	Median	Mean	Median
Carious								
LCA	0.1	0.1	4.6	3.7	4.0[a]	2.0[b]	0.5	0.0
HCA	24.6[a]	18.6[b]	7.8	2.2	1.0	0.0	5.2[a]	5.7
Caries-free								
CF	2.0	0.0	12.8	3.5	0.3	0.0	1.0	0.0
LCI	1.6	0.3	20.6[a]	16.0[b]	0.0	0.0	0.2	0.0
HCI	8.3	2.1	6.4	4.6	0.2	0.0	2.0	0.5
LCA	0.0	0.0	7.0	2.7	0.0	0.0	0.0	0.0
HCA	7.2	1.9	12.3	7.7	0.4	0.0	1.8	0.4

[a] Mean value significantly different from all other values in column; $P \leq 0.01$ (ANOVA, using Scheffe test).
[b] Median value significantly different from all other values in column; $P \leq 0.01$ (Mann-Whitney U test).

TABLE 4. *Changes in S. mutans proportions over time as a function of caries status of the tooth*

Caries status of tooth	Time before diagnosis (mo)					
	12		6		0	
	Mean	Median	Mean	Median	Mean	Median
Carious						
LCA (4)[a]	0.2	0.0	0.5	0.0	0.1	0.1
HCA (35)	6.5	0.2	9.5	1.0	24.6[b]	18.6[b]
Caries-free						
CF (18)	1.8[c]	0.0	0.8[c]	0.0	0.7[c]	0.0
LCI (28)	1.3[d]	0.5	2.8[d]	0.5	1.7[d]	0.3
HCI (22)	10.0	4.8	12.7	4.8	9.1	2.1
LCA (4)	0.1	0.0	4.5	0.0	0.0	0.0
HCA (60)	11.6	1.6	6.5	0.8	7.2	1.9

[a] Number in parentheses is number of comparisons for paired *t* test.
[b] □, Mean and median values significantly different from all other values in row and column; $P \leq 0.01$ (paired *t* test, Wilcoxon rank pair test, ANOVA, using Scheffe test, and Mann-Whitney *U* test).
[c] Mean values for CF group significantly lower than those found in HCI and HCA groups; $P < 0.01$ (ANOVA, using Scheffe test).
[d] Mean values for LCI group significantly lower than those in HCI group; $p \leq 0.01$ (ANOVA, using Scheffe test).

TABLE 5. *Changes in S. sanguis proportions over time as a function of caries status of the tooth*

Caries status of tooth	% *S. sanguis* at given time before diagnosis (mo)					
	12		6		0	
	Mean	Median	Mean	Median	Mean	Median
Carious						
LCA	8.4	2.6	3.4	2.8	4.6	3.7
HCA	13.8	8.6	9.8[a]	5.3	7.8	2.2
Caries-free						
CF	10.7	1.7	20.7	16.3	12.8	3.5
LCI	25.1[b]	24.7	15.7	12.7	20.2	16.0
HCI	5.7	2.7	10.1	6.0	6.4	4.6
LCA	17.4	16.9	6.0	0.3	7.0	2.7
HCA	12.4	7.9	16.4	10.0	12.2	7.7

[a] Significantly lower than caries-free fissures in HCA group; $P \leq 0.01$ (ANOVA, using Scheffe test).
[b] Significantly higher than in HCI group; $P \leq 0.01$ (ANOVA, using Scheffe test).

TABLE 6. *Changes in lactobacilli proportions over time as a function of caries status of the tooth*

Caries status of tooth	% Lactobacilli at given time before diagnosis (mo)					
	12		6		0	
	Mean	Median	Mean	Median	Mean	Median
Carious						
LCA	1.1	0.1	25.2	0.0	4.0[a]	2.0
HCA	0.0	0.0	1.1	0.0	1.0	0.0
Caries-free						
CF	0.0	0.0	0.0	0.0	0.3	0.0
LCI	0.0	0.0	0.0	0.0	0.0	0.0
HCI	0.1	0.0	1.0	0.0	0.3	0.0
LCA	0.1	0.0	0.8	0.0	0.0	0.0
HCA	0.0	0.0	0.1	0.0	0.4	0.0

[a] Significantly higher than all other mean values in column; $P \leq 0.01$ (ANOVA, using Scheffe test).

TABLE 7. *Changes in S. mutans/S. sanguis ratio over time as a function of caries status of tooth*

Caries status of tooth	Time before diagnosis (mo)					
	12		6		0	
	Mean	Median	Mean	Median	Mean	Median
Carious						
LCA	0.27	0.0	0.16	0.0	0.51	0.0
HCA	1.38[a]	0.02	2.02[a]	0.16	5.23[b]	5.66[b]
Caries-free						
CF	0.21	0.01	0.03	0.0	0.96	0.0
LCI	0.29[c]	0.02	0.61[c]	0.09	0.22[c]	0.02
HCI	3.64	1.60	3.25	0.5	2.0	0.46
LCA	0.01	0.0	1.10	0.0	0.0	0.0
HCA	1.98	0.23	1.35	0.06	1.84	0.4

[a] Significantly lower than −6-month value; $P \leq 0.05$ (paired t test).
[b, c] See footnotes *b* and *d*, respectively, Table 4.

TABLE 8. *Bacteriological status of caries-free and carious teeth in HCA patients (mean values)*

Bacteriological parameter	Time before diagnosis (mo)					
	12		6		0	
	Pre-carious	Caries-free	Pre-carious	Caries-free	Carious	Caries-free
Total count (× 10^5)	19.2[a]	13.1[a]	25.6	10.4	11.6[a]	4.6[a]
S. mutans (× 10^5)	1.0	0.3	0.6	0.6	1.1[a]	0.9[a]
S. sanguis (× 10^5)	2.1	1.0	2.7	0.9	3.2	0.4
Lactobacilli (× 10^5)	0.001	0.005	0.24	0.01	0.079	0.01
S. mutans (%)	6.5	11.6	9.5	6.5	24.6[b]	7.2
S. sanguis (%)	13.8	12.4	9.8[c]	16.4[c]	7.8[a]	12.2[a]
Lactobacilli (%)	0.0	0.01	1.1	0.04	1.0	0.4
S. mutans/S. sanguis	1.38	1.98	2.02	1.35	5.23	1.84

[a] Difference between values significant by Mann-Whitney U test, $P < 0.05$.
[b] Difference between values are significant by both Student t test and Mann-Whitney U test, $P < 0.01$.
[c] Difference between values significant by Student's t test, $P < 0.05$.

component, <1% of the fissure flora in the five teeth that became carious in the LCA subjects. *S. mutans* comprised about 10% mean value of the flora in caries-free fissures in the HCI subjects (median value, 2 to 4.8%) and was not a prominent member of the fissure flora in the CF, LCI, and LCA groups. *S. sanguis* proportions did not exhibit any significant changes over time in any of the fissures (Table 5), but there was a tendency for the proportions of this organism to be higher in caries-free fissures. The proportions of lactobacilli were low or zero in all subjects at all times except for the few carious fissures in LCA subjects (Table 6). Six months before the diagnosis of caries, lactobacilli accounted for 25% of the flora and then declined to 4% at the time caries was diagnosed. Too few teeth were available for this difference to be significant. The *S. mutans/S. sanguis* ratio confirmed the importance of *S. mutans* in the carious fissures of HCA subjects (Table 7). The mean value of 5.23 and median value of 5.66 found at the time caries was diagnosed differed significantly from the *S. mutans/S. sanguis* ratios found 6 and 12 months earlier in these fissures. No significant fluctuation in this ratio was observed in the fissures of the other groups. The median values appeared to be more specific than the mean values in analyzing the *S. mutans/S. sanguis* ratios in regards to caries occurrence; with one exception, the HCI group at −12 months, the only time the median ratio was over 1.0 was when caries was diagnosed in the HCA group. The mean values were greater than 1.0 in fissures in the HCI and HCA groups at all time periods.

The bacteriological differences between carious and caries-free fissures in the HCA subjects at all time periods were compared (Table 8). Twelve months before the diagnosis of caries, there were few differences in the bacteriological parameters under investigation between a fissure that became carious and one that remained caries-free. The total viable count was slightly but significantly higher in the pre-carious fis-

sures. Six months before the diagnosis of caries, the fissures that became carious had significantly lower proportions of *S. sanguis*. At the time the caries were diagnosed, the proportions of *S. mutans* and the *S. mutans*/*S. sanguis* ratio were significantly higher in the carious fissures (Student *t* test and Mann-Whitney *U* test, $P < 0.01$). The differences in the total count, *S. mutans* levels, and percentage of *S. sanguis* were significant (Mann-Whitney *U* test, $P < 0.05$). There were atypically high proportions of *S. mutans* and high *S. mutans*/*S. sanguis* ratios in some caries-free fissures. Table 9 demonstrates the *S. mutans* profile in four fissures monitored in one HCA subject. The patient entered the study at age 11.5 years, at which time he had 20 filled surfaces in his primary teeth and 9 filled surfaces in his permanent teeth. Tooth J, surface 6, was caries-free at entry. Six months later, this fissure was judged carious and had about 21% *S. mutans* and an *S. mutans*/*S. sanguis* ratio of 10. Subsequently, three premolars were added to the study as they erupted. These fissures had undetectable or low proportions of *S. mutans* on the first sampling and then showed a great increase in the proportions of *S. mutans* on the succeeding examinations, from 23 to 61%. These elevated proportions of *S. mutans* persisted over the next 1 to 2 years without any clinical decay. In fact, tooth 28, surface 6, has maintained both a high proportion of *S. mutans* and a high *S. mutans*/*S. sanguis* ratio for about 4 years without becoming decayed. During this time period, four surfaces on other teeth have become decayed; therefore, the patient correctly belonged in HCA group. The levels and proportions of *S. sanguis* and lactobacilli during this time period did not change. The decline in the caries score in the primary teeth reflected loss of teeth due to exfoliation.

DISCUSSION

The data obtained from 42 carious and 153 caries-free fissures strongly indicated an etiological role for *S. mutans* in most of the diagnosed fissure lesions. This was demonstrated by the longitudinal analysis, which showed the proportions of *S. mutans* and the *S. mutans*/*S. sanguis* ratio to increase significantly at the time that the earliest form of the carious lesion could be detected clinically (Table 4), and by cross-sectional comparisons, which showed that the proportions of *S. mutans* and the *S. mutans*/*S. sanguis* ratio in the carious fissures were significantly higher than in caries-free fissures (Table 3). This *S. mutans*-caries relationship was evident in other comparisons. The caries-free fissures in both the HCI and HCA subjects had significantly higher proportions of *S. mutans* than did the caries-free fissures in the LCI and LCA subjects, a phenomenon which agrees well with the significantly higher caries experience in these HCI and HCA subjects. These statistical differences could be shown by both parametric and nonparametric analyses and by dependent and independent statistical tests.

The present result confirms and extends the findings of many studies that implicate *S. mutans* as an important human odontopathogen (4, 8, 19, 23, 33, 38). In particular, they demonstrate that *S. mutans* is significantly involved in occlusal fissure decay, a finding suggested previously from cross-sectional studies (20, 23). This is of some clinical importance, since fissure decay

TABLE 9. *Atypical S. mutans pattern in an HCA subject*

Bacteriological parameter and tooth	Age of subject (yr)						
	11.5	12.0	12.5	13.1	13.7	14.3	16.2
S. mutans (%)							
J/6	6.4	20.7[a]					
28/6		0	24.1	53.8	51.1	37.9	43.6
5/5			1.8	61.4	55.0	17.3	0.3
12/5			0.2	23.3	44.0	30.3	7.3
S. mutans/*S. sanguis*							
J/6	0.55	10.0					
28/6		0	1.65	10.0	5.0	1.4	10.0
5/5			0.08	10.0	10.0	0.72	0.19
12/5			0.01	5.54	2.46	2.3	0.47
DFS							
Primary	20	16	10	10	7	—[b]	—
Permanent	9	10	10	10	12	14	14

[a] Caries diagnosed.
[b] —, Teeth lost due to exfoliation.

occurs before smooth-surface decay in most individuals and represents the most prevalent form of human tooth decay (1, 3). The pathogenesis of *S. mutans* in fissure decay may differ somewhat from that associated with smooth-surface decay, since glucan formation is not necessary for decay to occur (36). The ability of *S. mutans* to produce acid and to survive in a low-pH environment could be the main determinant of virulence in the fissure site (36; D. S. Harper and W. J. Loesche, unpublished data).

Although the results are striking in that they implicate *S. mutans* in fissure decay, they show that clinical decay can occur in the absence of *S. mutans*, as was observed in the LCA subjects. These LCA subjects were exceptional in that they had undetectable to low proportions of *S. mutans* in the 11 sampled fissures and high proportions of lactobacilli in the 5 fissures judged to become carious. Although there were few carious fissures, the proportions of lactobacilli found were still significantly higher than in the other fissures (Tables 3 and 7). In these LCA subjects, lactobacilli appeared to be specifically related to caries development.

The persistent moderately high mean proportions of *S. mutans* in the caries-free fissures of HCI and HCA subjects, i.e., 7 to 12% suggest that certain factors present in the fissure environment can in some instances modify the cariogenic potential of *S. mutans*. This was best illustrated by the data on the HCA subject shown in Table 9. Three premolars sampled shortly after their eruption had undetectable to low proportions of *S. mutans*. Approximately 6 months later, the proportions were elevated to levels that were consistent with caries development on teeth in the other individuals, and yet no decay occurred then or during the succeeding 3 years. This subject developed four new approximal lesions during this time period, suggesting that host and dietary factors contributing to caries (i.e., sucrose intake, susceptible tooth surfaces, absence of immune factors) were still operating in his mouth. Possible explanations for the non-cariogenicity of *S. mutans* in this subject could be the presence of other members of the fissure flora which (i) maintained a neutral or alkaline environment by producing base equivalents either from dietary components or from salivary products (17), which could eventually result in the mineralization of the fissure (7); (ii) converted lactic acid to weaker acids, such as *Veillonella alkalescens* was shown to do in gnotobiotic experiments (27); (iii) hydrolyzed the extracellular polymers of *S. mutans* (31); or (iv) simply remained transposed between *S. mutans* and the enamel, thereby spatially occluding *S. mutans* and its products from the susceptible tooth surfaces. Alternately, the *S. mutans* in these subjects could have been of reduced or absent virulence as a result of mutations (29, 36) or of genetic variation between genotypes of *S. mutans* (25). Two additional HCA or HCI subjects exhibited this atypically high *S. mutans*-no caries pattern, giving an observed frequency of occurrence of 3 out of 35 subjects. This suggests that a diagnosis of fissure caries based on high proportions of *S. mutans* may be incorrect in at least 10% of the subjects.

The present results are in apparent disagreement with the results of an ongoing English investigation of the flora associated with the incipient carious lesion on the distal approximal surface of the first maxillary premolars (2, 11). The two studies differed in their choice of tooth surfaces to be sampled, the sampling procedure itself, and the subsequent manipulation of the plaque samples. Any of these factors alone or in part could explain the observed differences.

The choice of site for plaque collection is perhaps of paramount importance in a clinical caries study. Since *S. mutans* levels and proportions on different teeth in the same mouth (37), on a given tooth (28), or within a given tooth site such as a buccal-surface white spot (5) can vary by a factor of 10 or more, discrete sites must be sampled in a standardized manner. Plaques were removed from occlusal fissures in the present study because fissures tend to be the most caries-prone sites on a tooth (1, 3). In the English study (2, 11), the distal surface of the maxillary first premolar was sampled. This choice of site would allow maximum standardization of the radiographs used for caries diagnosis and would facilitate the collection of plaque samples. This site difference could in part explain the different results of the investigations.

The studies differed in the method of removing plaque from the tooth surface. In this investigation, a standardized needle with a tip approximately 0.1 mm in diameter was forcibly scratched along the entire length of the fissure. Only fissure plaque was removed, even though the sample would be a mixture of plaque removed from parts of the fissure that were developing decay and parts that were not. In the English study, a standardized abrasive strip about 2 mm wide was soldered to a handle. The strip was inserted beneath the contact point (the caries-prone site) and used to remove tooth surface plaque (2). Our experience with this method indicates that it is an effective means for removing plaque, but that supragingival plaque at the gingival margin is removed along with the plaque from beheath the contact point. If so, then this sampling procedure would dilute the sought-after cariogenic flora by including high

numbers of gingival organisms such as *Bacteroides* spp. This assumption is supported by the observation that gram-negative anaerobic rods, including *Bacteroides melaninogenicus*, constituted about 20% of the isolates, whereas *S. mutans* was usually less than 1% (11). Also, if *S. mutans* invades the tooth, either into the white spot (22) or into a beginning cavitation, the abrasive strip may pass over this lesion and not remove the bacteria at the advancing margin of the caries front.

The plaques in the present investigation were dispersed by sonication and were cultured on MM10 sucrose medium. The plaques in the English study were dispersed mechanically with a tissue grinder and cultured on a blood sucrose medium known to support *S. mutans* (34). To determine whether the different methodologies could account for this difference in plaque composition, the media and dispersal procedures were compared (G. H. Bowden and W. J. Loesche, unpublished data). The results obtained from several plaque samples showed the procedures to be equivalent, suggesting that the difference in *S. mutans* proportions between the investigations was due to different plaque samples and not to different laboratory procedures.

Plaque or salivary levels of both *S. mutans* and lactobacilli have been suggested (35) or used to diagnose (17, 22) patients with a high caries risk. In the present investigation, high levels of plaque lactobacilli clearly identified in the LCA subjects the fissures destined to become carious (Table 6). High proportions of *S. mutans* identified the subjects with a high caries experience but seemed less able to predict which fissures would become decayed, given the criterion for decay used, i.e., a catch with the dental explorer. However, in most dental public health examinations and most clinical practices, these occlusal catches would not be considered carious. The present results indicate that in 37 such catches a significant increase in *S. mutans* occurred and that in five others a significant increase in lactobacilli was detected. These findings justify some form of professional intervention at the catch stage, since the dominance of organisms with a known odontopathic potential cannot be ignored. This intervention does not have to be amalgam restoration as was used in the present investigation, but could include dietary counseling (18), antimicrobial treatment (18, 22) or the placement of sealants (9). The significant elevation of *S. mutans* found coincident with a catch may indicate an early lesion that is amenable to treatment methods less destructive than an amalgam restoration.

The CF subjects were significantly younger at entry into the study than the subjects in the other groups, which could account in part for their caries-free status. Their proportions of *S. mutans* were significantly lower than those found in the carious fissures of the HCA subjects and the caries-free fissures of HCI and HCA subjects (Table 4); in this regard they resembled the LCI and LCA subjects. Therefore, it may be possible on the basis of their *S. mutans* profile to predict that the future caries experience of these CF subjects will be low (38). These subjects will continue to be monitored.

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