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Longitudinal Investigation of Bacteriology of Human Fissure Decay: Epidemiological Studies in Molars Shortly After Eruption

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In the present investigation, the proportions of *Streptococcus mutans*, lactobacilli, *Streptococcus sanguis*, veillonellae, and an unidentified actinomyces-like organism in dental plaque on occlusal fissures of first mandibular molars were monitored at 6-month intervals over a 3-year period in 368 children who were initially in grades 1 or 2. Teeth destined to become decayed exhibited a significant increase in the proportions of *S. mutans* from 6 to 24 months before the diagnosis of dental decay. Lactobacilli were sporadically detected but when present were associated with dental decay. Children whose teeth exhibited the greatest number of decayed surfaces had, at all time periods, significantly higher proportions of *S. mutans* than did children who were caries free. Many teeth had high proportions of *S. mutans* at their entry into the study. About 10% of the monitored teeth erupted during the period of observation, and in these teeth both *S. mutans* and lactobacilli could be significantly associated with decay. In these newly erupted teeth *S. mutans* outnumbered lactobacilli by ca. 20 to 1. *S. sanguis*, veillonellae, and the unidentified actinomyces-like organism could not be associated with the development of decay. These findings strongly implicate *S. mutans* and possibly lactobacilli as dental pathogens and suggest that if decay is to be controlled by strategies based upon a *S. mutans* infection, then the various tactics used probably will have to be performed on primary teeth, as these teeth are the most likely sources of infection for the permanent teeth.

The demonstration of bacterial specificity in a chronic infection such as dental decay is difficult, especially when the suspected etiological agent, *Streptococcus mutans*, appears to be present on all dentitions (2, 19). Cross-sectional association studies indicate that *S. mutans* is significantly elevated in levels or proportions, or both, in the dental plaques taken from decayed (D) sites, as compared with plaques removed from caries-free (CF) sites on the same (5, 23) or different teeth (4, 16, 19, 20, 26).

Association studies cannot distinguish whether the proportional overgrowth of *S. mutans*, and to a lesser extent lactobacilli, is the cause or the result of the dental decay. Longitudinal studies presumably would demonstrate whether the increase in these organisms coincided with or predicted the development of clinical lesions. Longitudinal investigations support a role for *S. mutans* in the development of decay (1, 2, 8, 10, 11, 21, 30), but it is clear that some lesions can occur in the absence of detectable *S. mutans* (8, 21) and that some teeth can have high proportions of *S. mutans* without becoming carious (21). These investigations, with the exception of the study of patients with postradiation xerostomia (1), did not necessarily study the subjects or the tooth surface most at risk to caries, i.e., the occlusal fissures of molar teeth shortly after their eruption (3, 17). In the present investigation, plaque was cultured from the occlusal fissures of first mandibular molars shortly after their eruption to determine the relationship between proportions of selected plaque species and the diagnosis of dental caries. A population of children initially in grades 1 and 2, residing in a nonfluoridated community, was sampled at 6-month intervals over a 3-year period. Data obtained after 2 years and restricted to those children who were present for each examination showed that *S. mutans* was endemic in these children, that its levels were stable, and that high levels of infection were associated with the development of caries (2). The present report expands these findings by giving 3-year data on all children analyzed as a function of the caries status of the patient and the tooth, using the date of diagnosis of decay as the reference time (21). In addition, information is provided on the proportions of lactobacilli, veillonellae, an unidentified actinomyces-like organism, and *Streptococcus sanguis* as well as *S. mutans*.

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MATERIALS AND METHODS

Subjects. All children attending grades 1 and 2 in the Coldwater, Mich., school system (population of Coldwater, 8,000) were given information concerning the prospective clinical study in the fall of 1978. Children whose parents consented to their participation in the study were examined initially in April 1979 and at 6-month intervals thereafter until April 1982. The Coldwater school system was chosen because of the absence of community water fluoridation (0.2 ppm [μg/ml] of fluoride), which gives these children a higher caries rate than those in neighboring fluoridated communities.

Clinical examination. Dental explorer examinations for dental decay were performed annually for the entire dentition and biannually for the first molars by the same dentist. The criterion for caries was that softness or a definite break in the continuity of the enamel surface had to be detected with the explorer (2).

Bacteriological procedures. The number of subjects involved (495 at base line) and the number of plaques samples (ca. 1,000 at base line) required that each biannual sampling period consist of 10 to 15 collection days spread over a 3- to 4-week period. Approximately 40 to 50 subjects were examined each morning, and 80 to 100 fissure plaques were removed and cultured by methods previously described (2, 21). Briefly, plaque samples were obtained from the occlusal fissures of the lower first molars with a no. 26-gauge sterile needle, held with a hemostat. Each needle which contained from 10^3 to 10^7 CFU of bacteria was dropped into a reduced transport fluid (20) and transported back to the laboratory.

TABLE 1. Population groups based upon caries status

Group	No. of subjects who finished	Age (mo) at start	Caries score[a]			
			Primary		Permanent	
			At start	At finish	At start	At finish
CF	70	89.9	0	0	0	0
Caries inactive						
LCI	20	90.4	1.8	1.8 (0)	0.4	0.4 (0)
HCI	7	89.4	10.6	10.6 (0)	0.3	0.3 (0)
Caries active						
LCA	95	91.5	0.4	1.0 (0.6)	0.1	0.7 (0.6)
HCA	176	90.4	7.6	11.5 (3.9)	0.3	4.3 (4.0)

[a] Caries score reflects the number of D, M, and F surfaces (DMFS score) in the primary or permanent dentition at the start and at the finish of the period of observation. The number within parentheses is the number of new carious surfaces observed.

No samples were taken from teeth that had a dental restoration.

The individual plaque samples were dispersed for 5 s, serially diluted, and plated by means of a semiautomated plating device (Spiral Systems Inc., Cincinnati, Ohio) onto MM10 sucrose agar for total viable counts and for *S. mutans* and *S. sanguis* counts (20); onto LBS agar for total lactobacilli counts (25); onto modified MM10 agar without added carbohydrates but containing 0.6% lactate, 5 μg of vancomycin per ml, and 4 μg of bromothymol blue per ml for veillonellae counts (24); and onto MSB agar, a selective medium, for *S. mutans* (6). Nonpigmented colonies of an actinomyces-like organism could be reliably recognized on the MM10 sucrose agar, and counts of this organism were also recorded. This organism was an anaerobic, saccharolytic, catalase-negative, gram-positive pleomorphic rod and was not further characterized.

All plates were incubated for 5 to 7 days under an atmosphere of 85% N_2–10% H_2–5% CO_2 in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mich.). All colonies which appeared on appropriately diluted MM10 sucrose plates were enumerated, as well as the colonies of *S. mutans*, *S. sanguis* (adherent colonies), lactobacilli, veillonellae, and the actinomyces-like organism. The *S. mutans* counts on the MSB medium were recorded, but as they invariably were lower than the counts on the MM10 sucrose agar (20, 21), they were only used in the calculations when it was difficult to detect *S. mutans* on a crowded MM10 sucrose plate. The proportions of *S. mutans*, *S. sanguis*, veillonellae, lactobacilli, and the actinomyces-like organism were obtained by dividing the counts of these organisms by the total count on the same or corresponding MM10 sucrose plate. No proportions were calculated when less than 20 CFU were present on the plate upon which the total count was obtained. This procedure eliminated distortion of the data, which can occur when calculations are based upon a small total count in the denominator (21).

Statistical analysis. The participants were stratified into five groups, based upon their prior dental caries experience, i.e., the number of D, missing (M), and filled (F) tooth surfaces present at the outset of the study, and upon their caries experience during the 3-year period of observation. A DMF tooth-surface score of 5 was used to partition between individuals with low and high initial caries activity, and the detection of a caries lesion or the presence of a filling during the period of observation classified the subject as caries active (21). This scheme separated the present population into five groups, i.e., a CF, low caries-inactive (LCI), high caries-inactive (HCI), low caries-active (LCA), and high caries-active (HCA) groups (Table 1). The monitored clinical and bacteriological parameters were evaluated in these groups at each time period by an analysis of variance with a pairwise *t* test, utilizing pooled within group mean squares to compare differences between groups.

Some children were seeing their private dentist independent of this study, and when these dentists detected decay, a dental filling was placed. It is assumed that the private dentists used variable criteria to diagnose decay and that these criteria were not necessarily the same as the study dentist. Because of this difference in the diagnosis of caries, the data obtained from the teeth that were F (filled) by private dentists are analyzed separately from those that were diagnosed as D (decayed) by the study dentist. The proportions of the monitored bacterial species were calculated and compared at the time of caries diagnosis or placement of a filling, with the corresponding values obtained at the prior time periods. This was accomplished by the use of a paired *t* test and by a nonparametric ranking procedure (Wilcoxon test) that can be used with paired data.

RESULTS

Data were analyzed from 368 children who remained in the study during the entire observational period or who exited from the study because either dental fillings were placed or caries was diagnosed in both mandibular molars. Seventy of these children were CF at the start of the study and remained that way throughout the observation period (Table 1). Of the 115 children who were categorized as exhibiting a low caries experience before the start of the study, 20 became caries inactive during the period of observation, whereas 95 developed an average of 1.2 new lesions (Table 1). Of the 183 children who were categorized as exhibiting a high caries experience before the start of the study, 7 became caries inactive, whereas the remaining 176 children went on to develop an average of 7.9 new lesions (Table 1).

The presence of a filling is an indisputable observation, whereas ca. 1% of the clinical diagnoses of decay were reversed on subsequent visits (2). Only those teeth, which were diagnosed as D on two successive 6-month examinations, or were diagnosed as decayed at one examination and replaced with a filling on the next examination, were considered as D in the statistical analysis. A total of 132 teeth were diagnosed as D, and 161 teeth had dental fillings placed without a prior diagnosis of decay by the study examiner.

The exit of these 293 teeth from the study was evenly distributed over the 36-month observation period. A total of

TABLE 2. *S. mutans* proportions

Caries status		*S. mutans* proportions[a] (%) at: Time of diagnosis		Time (mo) before diagnosis of caries or placement of filling: 6		12		18		24		30	
Patient	Tooth[b]	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med
LCA	D (23)	25.6[c]	19.2	22.7[d]	19.6	14.6	13.6	16.8	6.9	14.8	1.8	10.8	1.3
	F (44)			18.1[d]	4.3	11.3	3.6	15.3[d]	5.1	9.8[d]	2.7	13.3	6.1
	CF (111)	11.2	4.2	12.8	5.3	15.2[d]	3.8	12.8[d]	2.1	9.3[d]	2.2	10.1	1.3
HCA	D (109)	24.3[c]	17.0	25.7[c]	22.2	25.9[c]	22.4	25.6[d]	18.5	21.3[d]	17.5	15.4[d]	9.2
	F (117)			18.1[d]	13.4	17.7[d]	8.1	16.7[d]	10.9	15.9[e]	7.9	15.0	10.0
	CF (147)	12.3	5.4	15.2[d]	6.5	17.3[d]	10.3	14.6[d]	6.8	14.8[de]	5.1	13.9[d]	4.6
CF	CF (132)	9.3	2.1	8.8[d]	2.3	9.7[d]	2.3	6.6[c]	1.8	7.1[de]	1.0	8.5[d]	2.0
LCI	CF (39)	9.8	3.2	12.1	7.2	12.9	5.7	15.8[d]	4.7	15.0[e]	3.6	14.1	3.4
HCI	CF (14)	7.5	3.4	15.2	7.5	15.2	5.6	18.3	12.9	16.1	8.3	18.5	3.4
Avg[f]		13.9 (573)		16.1 (698)		16.0 (643)		14.7 (590)		12.6 (531)		12.1 (446)	
Analysis of variance		$P < 0.0001$		$P < 0.0001$		$P < 0.0001$		$P < 0.0001$		$P < 0.0001$			

[a] Proportions are measured over time as a function of caries status of the patient and the tooth. Mean values are for the 6- to 30-month period before the diagnosis of decay or placement of a filling and for CF teeth. Med, Median value.
[b] The values within parentheses are the number of teeth. CF values were obtained in the same time frame as teeth destined to be D.
[c] Values are significantly different ($P < 0.05$) by the pairwise t test from all other values within the column.
[d] Values that are underlined are significantly different ($P < 0.05$) by the pairwise t test from other values within the column that are marked by a superscript *d*.
[e] Values that are underlined are significantly different ($P < 0.05$) by the pairwise t test from other values within the column that are marked by a superscript *e*.
[f] Values within parentheses are number of teeth.

290 teeth were available for comparison purposes for the 6-month visit that immediately preceded exit, whereas 246 teeth were available for the 12-month comparison. This number declined at each antecedent visit, so that for the 36-month visit only 39 teeth were available for comparison purposes. As these 39 teeth gave rise to low numbers in the various statistical cells, and the findings provided no new information, this time period was omitted from the data tables which follow.

S. mutans was detected in the plaque flora of every child at some time during the period of observation. In those fissure surfaces that developed decay, the proportions of *S. mutans* averaged 25% of the cultivable flora in the LCA subjects and 24% in the HCA subjects at the time that decay was diagnosed (Table 2). Both values were significantly higher than the corresponding *S. mutans* proportions observed in the same time frame in the CF teeth, found either within the same mouth as the D teeth or in the CF or caries-

TABLE 3. Lactobacilli proportions

Caries status		Lactobacilli proportions[a] (%) at: Time of diagnosis		Time (mo) before diagnosis of caries or placement of fillings: 6		12		18		24		30	
Patient	Tooth[b]	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med
LCA	D (23)	7.1[c]	0.3	3.8	0.2	6.3[c]	1.7	0.4[c]	0.2	0.6	0.1	0.1	0.03
	F (44)			1.2[c,d]	0.2	1.0[c]	0.2	3.3[c]	0.3	1.2	0.1	1.1	0.4
	CF (110)	2.9[c,d]	0.4	1.6[c,d]	0.2	1.4[c]	0.3	0.8[c,d]	0.2	0.8	0.2	0.9	0.1
HCA	D (110)	5.2[d]	0.8	4.2[c]	0.3	1.8[c]	0.3	1.7[c]	0.2	1.0	0.1	0.3[c]	0.1
	F (117)			4.1[d]	0.4	3.8	0.3	2.5[d]	0.4	1.3	0.3	1.5[c]	0.1
	CF (146)	1.7[c,d]	0.2	1.1[c,d]	0.2	2.6	0.4	0.9[c,d]	0.1	0.8	0.2	0.8	0.3
CF	CF (130)	1.6[c,d]	0.2	1.3[c,d]	0.1	1.4[c]	0.2	0.6[c,d]	0.2	1.0	0.1	0.8	0.3
LCI	CF (39)	1.6[c,d]	0.3	1.2[c,d]	0.3	2.0	0.3	0.3[c,d]	0.2	0.8	0.1	0.4[c]	0.2
HCI	CF (14)	3.4	0.1	0.7	0.2	4.2	0.1	0.4[c]	0.2	0.7	0.3	0.4	0.1
Avg[e]	(572)	2.8		2.3 (695)		2.3 (644)		1.2 (591)		0.9 (529)		0.8 (447)	
Analysis of variance		$P < 0.002$		$P < 0.003$				$P < 0.001$					

[a] See footnote *a* of Table 2.
[b] See footnote *b* of Table 2.
[c] Values that are underlined are significantly different ($P > 0.05$) by the pairwise t test from other values within the column that are marked by a superscript *c*.
[d] Values that are underlined are significantly different ($P > 0.05$) by the pairwise t test from other values within the column that are marked by a superscript *d*.
[e] Values within parentheses are number of teeth.

TABLE 4. Proportion of *S. sanguis* over time in HCA and CF subjects

Caries status		Proportion[a] (%) of *S. sanguis* at:											
		Time of diagnosis		Time (mo) before diagnosis of caries or placement of filling									
				6		12		18		24		30	
Patient	Tooth	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med
HCA	D	5.2	1.6	4.8	2.1	5.7	2.9	5.6	1.8	9.6	3.4	8.4	2.4
	F			6.0	2.8	5.8	2.3	7.7	3.0	6.0[b]	3.2	5.9	2.9
	CF	4.4[b]	1.5	7.1	3.9	7.3	3.0	7.6	3.6	7.9[b]	5.2	6.1	2.9
CF	CF	7.0[b]	3.0	8.1	4.6	6.8	4.3	6.4	3.4	11.0[b]	5.4	7.3	3.5

[a] Mean values are for the 6- to 30-month period before the diagnosis of decay and for CF teeth. Med, Median value.
[b] Values that are underlined are significantly different ($P < 0.05$ by the pairwise t test from other values within the column that are marked by a superscript *b*.

inactive individuals. The lower proportions of *S. mutans* on sound teeth was particularly evident in the CF individuals, in that *S. mutans* never averaged more than 10% of the flora and the median values were never more than 2% over the entire period of observation. This value in the CF subjects was often significantly lower than the corresponding values found in sound teeth in the caries-active individuals (Table 2).

The mean and median proportions of *S. mutans* in the plaques removed from teeth destined to become D in the LCA individuals increased significantly between the 6th and 12th months before the diagnosis of decay ($P < 0.05$ by the paired t test) (Table 2). However, in teeth destined to become carious in the HCA individuals, the mean proportions of *S. mutans* increased significantly in the interval between 24 and 30 months before the diagnosis of decay (Table 2). Plaque removed from teeth that exited from the study because of the placement of a dental filling had, in the time periods preceding exit, mean proportions of *S. mutans* that were intermediate between values found on teeth that were diagnosed as D or CF (Table 2). These proportions were usually significantly higher than those found in plaques taken from CF fissures in CF individuals. In the LCA individuals, *S. mutans* proportions on the teeth destined to be filled and on those that remained CF were similar. However, the increase in *S. mutans* that occurred on the F teeth between 6 and 12 months was significant ($P < 0.05$ by the paired t and Wilcoxon tests).

Lactobacilli were sporadically detected in the plaques during the period of observation. When present, they tended to be found in plaques removed from teeth that were destined to become D or F. At the time of diagnosis of decay, lactobacilli averaged 7% of the cultivable flora in the plaques taken from the LCA subjects and 5% in the plaques taken from the HCA subjects (Table 3). Both values were significantly higher than the corresponding values found in the CF teeth in all but the HCI subjects (Table 3). The median values at the time of diagnosis of decay were low, being 0.8% in the HCA subjects and 0.3% in the LCA subjects. The 0.8% value was significantly higher ($P < 0.05$ by the Wilcoxon test) than the median values observed on these teeth at 12, 18, 24, and 30 months (Table 3).

Teeth destined to be F in the HCA subjects showed several significant relationships in regard to lactobacilli. The proportions of lactobacilli on these teeth at 6, 18, and 30 months were significantly higher than the values found on most teeth that would remain CF (Table 3). Also, the mean proportion of 4.1% observed at 6 months was significantly higher than the corresponding values observed on these same teeth at 18, 24, and 30 months ($P < 0.05$ by the paired t test).

S. sanguis, veillonellae, and the actinomyces-like organism represent indigenous plaque bacteria whose status relative to caries initiation is not known. The proportions of *S. sanguis* and the actinomyces-like organism tended to be lower in plaques taken from teeth destined to become D or F, whereas the proportions of veillonellae were relatively stable on these teeth. These patterns can be demonstrated by comparing the HCA and CF individuals (Tables 4, 5, and 6). At all time periods, the proportions of *S. sanguis* on the CF teeth in the CF subjects were higher than on the teeth in the HCA subjects. At the time of caries diagnosis, the D fissures had significantly lower proportions of the actinomyces-like organism relative to the CF fissures in the CF subjects (Tables 4 and 6).

In the CF teeth, especially those in the HCA individuals, the proportions of veillonellae were significantly reduced compared with the D teeth at the time of caries diagnosis and at 6 months before this diagnosis (Table 5).

S. mutans and *S. sanguis* exhibit an inverse relationship to each other in the dental plaque (4, 20). A previous investigation had shown that when the *S. mutans* to *S. sanguis* ratio

TABLE 5. Proportion of veillonellae over time in HCA and CF subjects

Caries status		Proportion[a] (%) of veillonellae at:											
		Time of diagnosis		Time (mo) before diagnosis of caries or placement of filling									
				6		12		18		24		30	
Patient	Tooth	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med
HCA	D	5.0[b]	1.1	4.3	1.1	9.1[b]	3.0	6.4	2.4	7.5	2.9	5.0	1.7
	F			5.5	1.7	5.9	2.7	6.5	1.9	6.2	2.0	11.6	4.4
	CF	0.8	0.2	1.5[b]	0.3	4.3	1.6	10.2	5.5	3.1	0.8	11.0	6.1
CF	CF	1.4	0.3	4.1	0.6	5.7	1.6	9.3	3.6	4.8	1.5	8.9	4.4

[a] Mean values are for the 6- to 30-month period before the diagnosis of decay and for CF teeth. Med, Median value.
[b] Values are significantly different ($P < 0.05$) by the pairwise t test from other values within the column.

TABLE 6. Proportion of an actinomyces-like organism overtime in HCA and CF subjects

Caries status		Proportion[a] (%) of an actinomyces-like organism											
		Time of diagnosis		Time (mo) before diagnosis of caries or placement of filling									
				6		12		18		24		30	
Patient	Tooth	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med	Mean	Med
HCA	D	2.6	0.3	2.7	0.3	3.8	0.6	3.8	0.3	5.8	1.3	6.5	1.4
	F			3.2	0.3	2.7	0.8	5.8	0.5	6.5	0.5	5.0	2.0
	CF	3.3	0.3	3.6	0.8	2.8	0.2	4.6	1.2	5.5	1.2	5.2	2.0
CF	CF	6.1[b]	0.8	3.9	1.0	4.7	0.6	4.4	1.2	6.0	1.8	6.4	1.9

[a] Mean values are for the 6- to 30-month period before the diagnosis of decay and for CF teeth. Med, Median value.
[b] Values are significantly different ($P < 0.05$) by the pairwise t test from other values within the column.

(MSR) was greater than 5.0, this ratio could be statistically associated with the earliest sign of a clinical lesion, i.e., the catch of a dental explorer in the occlusal fissure (21). The MSR was calculated individually for each plaque sample and reported as a positive integer if *S. mutans* was greater than *S. sanguis* and as a negative integer if *S. sanguis* was greater than *S. mutans*.

The mean MSR was significantly higher in those teeth destined to become D in the HCA subjects at five of the six time intervals compared with the teeth in the other subjects (data not shown). In fact, the HCA subjects differed from the other subjects in having essentially positive MSRs at all time intervals, i.e., on all occasions for teeth destined to become D or F and on five of six occasions for teeth which remained CF. In contrast, the MSR was negative on all occasions in the CF teeth in the CF subjects. The CF teeth in the LCA, LCI, and HCI subjects fluctuated between a positive and negative MSR. Teeth destined to become D in the LCA subjects exhibited a positive MSR only in the 12-month period which preceded the diagnosis of decay. Teeth which became F in the LCA subjects had mainly negative MSRs. Teeth destined to become D showed a significant increase in the MSR some 18 to 24 months before the diagnosis of decay in the HCA subjects and 6 to 12 more months before diagnosis of decay in the LCA subjects.

TABLE 7. Proportion of *S. mutans* and MSR

Caries status		At start of longitudinal investigation		At time of diagnosis of decay in LCA and HCA subjects	
Patient	Tooth	% *S. mutans*	MSR	% *S. mutans*	MSR
LCA	D	18.0[a]	−3.9[a]	25.6[b]	10.1
	F	12.1	7.5[a]	18.1	
	CF	10.8	10.4[a]	11.2	4.2
HCA	D	23.0[b]	8.6[a]	24.3[b]	19.9[b]
	F	15.3[a]	2.8	18.0	
	CF	12.7[a]	−2.9[a]	12.3	10.2
CF	CF	6.7[a]	−16.1[a]	9.3	−0.5
LCI	CF	11.2	−2.8[a]	9.8	4.4
HCI	CF	2.7	−18.9[a]	7.5	−0.9
Analysis of variance		13.1 $P < 0.001$	−4.5 $P < 0.0001$	13.9 $P < 0.0001$	7.7 $P < 0.0001$

[a] Values that are underlined are significantly different ($P < 0.05$) by the pairwise t test from other values within the column that are marked by a superscript [a].
[b] Values are significantly different ($p < 0.05$) by the pairwise t test from other values within the column.

The proportions of *S. mutans* and the MSR for all subject categories at the start of the investigation and at the time of the diagnosis of decay are shown in Table 7. The teeth destined to develop decay in the HCA subjects had a cariogenic level of *S. mutans* and an elevated MSR at the outset of the study, despite the fact that this study was initiated within 1 to 15 months after tooth eruption.

The relationship of this prior infection to caries development was determined by analyzing the *S. mutans* parameters in the teeth which became D, at each 6-month time interval measured from the start of our investigation (teeth which received a dental filling were excluded). Teeth which became D within 6 and 12 months from the start of the study had 30% *S. mutans* and a MSR above 14 at the outset (Tables 8 and 9). Teeth which became D after 18 months had 28% *S. mutans* and a MSR of 6 at the outset. For all of these teeth the MSR increased to 20 within the first 6 months. Teeth which became D after 24 to 30 months had initially lower proportions of *S. mutans* and a negative MSR. Both *S. mutans* parameters increased to cariogenic values within the first 6 months of observation, and the teeth were diagnosed as D 18 to 24 months later (Tables 8 and 9). Teeth which became D after 36 months had moderate proportions of *S. mutans* and low MSRs during the fist 18 to 24 months of observation. Then the proportions of *S. mutans* and the MSR increased between the 24- and 30-month period of observation, and within 6 months, these teeth were found to be D.

This analysis indicates that teeth in the HCA subjects destined to become D within the first 6 to 18 months of our observation period entered the study with a cariogenic load of *S. mutans*. Those teeth, not so encumbered initially with high proportions of *S. mutans*, also acquired a cariogenic

TABLE 8. Change in *S. mutans* as a function of time in which decay was diagnosed

Decay diagnosed at month[a]	Change[b] in MSR at time (mo) from start of study						
	0	6	12	18	24	30	36
6 (16)	19.2	22.3					
12 (16)	14.3	22.1	17.5				
18 (21)	6.6	19.9	13.7	13.2			
24 (14)	−3.6	11.6	7.5	14.2	5.5		
30 (19)	−1.7[c]	⇀14.4	15.9	11.6	26.3	13.3	
36 (13)	−2.4	5.3	2.2	7.7[d]	10.2	16.7[d]	26.8[c]

[a] Values within parentheses are number of teeth.
[b] ⇀, Values are significantly different by the paired t test.
[c] Value is significantly different ($P < 0.05$) by the paired t test from other values in the row.
[d] Values are significantly different ($P < 0.05$) by the paired t test.

TABLE 9. Change in MSR as a function of time in which decay was diagnosed

Decay diagnosed at month[a]	Change[b] in % *S. mutans* at time (mo) from start of study						
	0	6	12	18	24	30	36
6 (16)	30.2	20.3					
12 (16)	29.8	26.0	28.7				
18 (22)	28	23.8	19.4	24.7			
24 (13)	8.7	19.5	13.1	15.2	19.1		
30 (19)	12.7 ⇀	30.6	29.2	23.0	30.6	26.3	
36 (15)	15.8	18.3	16.0	16.7[c]	17.7	29.3[c]	34.7

[a] Values within parentheses are number of teeth.
[b] ⇀, Values are significantly different ($P < 0.05$) by the paired t test.
[c] Values are significantly different ($P < 0.05$) by the paired t test.

load of *S. mutans* and became D within 6 to 24 months after this overgrowth occurred.

Approximately 10% of the investigated molars erupted after our first examination. As such, these teeth represented a unique subset of teeth in which to observe the temporal relationship between caries development and the proportions of the monitored bacterial species. A total of 10 of these teeth eventually were diagnosed as D, 15 were F, and 45 remained CF during the period of observation.

The D teeth at the time of diagnosis had, relative to the CF teeth in the same time frame, significantly higher proportions of *S. mutans*, i.e., 29 versus 8.5%, and lactobacilli, i.e., 8.3 versus 1.9% (Tables 10 and 11). There was a clear incremental increase in percent *S. mutans* in the 12-month period before the diagnosis of decay. No such pattern was observed in the teeth destined to be F or in the teeth which remained CF during this period of observation. The percent lactobacilli increased sharply 6 months before the diagnosis of decay and tended to increase in the teeth destined to be F (Table 11).

The median values for *S. mutans* at all time intervals were ca. 20 to 30 times greater than the lactobacilli values, i.e., 4.1 to 7.9% *S. mutans* versus 0.12 to 0.26% lactobacilli. The percent *S. mutans* was consistently above the median value at 0, −6, and −12 months in those teeth destined to become D and usually below the median value in the CF teeth. No pattern would be discerned in the teeth destined to become F. The percent lactobacilli was above the median value in both the D and F teeth at 0 and −6 months and tended to be below the median value in the CF teeth.

DISCUSSION

The longitudinal data obtained from ca. 700 initially CF occlusal fissures demonstrate that when *S. mutans* gains ascendency in the plaque flora, dental decay is a probable consequence. This etiological relationship was shown, despite the difficulties encountered when attempting to show bacterial specificity in an infection in which other bacteria are present and in which the natural history of the infection may take months before the multiple episodes of demineralization and remineralization of the tooth surfaces proceed to the stage at which the infection can be detected clinically (27).

The time course of the infection can be influenced by changes in the diet, especially as it relates to between-meal eating of sucrose (7, 19), varying degrees of exposure to fluoride (18), the medical usage of antibiotics which can suppress the salivary levels of *S. mutans* for several weeks (22), the salivary *S. mutans* levels of family members (14, 29), and possibly oral hygiene habits and dental treatments

TABLE 10. Mean proportions of *S. mutans* in teeth

Time (mo) before diagnosis	Mean proportions (%) of *S. mutans* in teeth[a]		
	D	F	CF
0	29[b] (10)		8.5[b] (45)
6	25.1 (10)	14.9 (15)	17.2 (44)
12	16.2 (8)	19.7 (14)	9.4 (39)
18	8.7 (6)	15.5 (11)	10.6 (41)

[a] Mean proportions are measured as a function of time before the diagnosis of decay in teeth that erupted during the period of observation. Values within parentheses are the number of teeth.
[b] Values with the same superscript are significantly different ($P < 0.05$) by the Scheffe test.

(19). In addition, there may be salivary factors related to both composition and flow (12), microbial interactions in the plaque, and host immunological experience that could affect the progression of the infection. This formidable list of complicating factors probably assures that no unequivocal demonstration of the etiological role of *S. mutans* in human decay will be forthcoming.

The present investigation sought to overcome some of these confounding factors by culturing at 6-month intervals the plaque flora present in the most caries-prone tooth site, i.e., the occlusal fissure; in the most caries-prone tooth, i.e., the mandibular first molars; and in the time span in which these teeth are most prone to decay, i.e., in the years immediately after eruption (3, 17). To assess the role of bacterial species other than *S. mutans*, the levels of lactobacilli, *S. sanguis*, veillonellae, and an actinomyces-like organism were monitored. These organisms, with the exception of the lactobacilli, were detected in ca. 80% of the ca. 6,000 plaque samples that were cultured, and, accordingly, could be considered indigenous plaque species (19).

The proportions of these monitored bacterial species were relatively stable over the 3-year observation period, except for the increases in proportions of *S. mutans* and lactobacilli in those teeth destined to develop decay (Tables 2, 3, 10, and 11). These data indicate that increases in *S. mutans* to ca. 20 to 25% (mean value) of the cultivable flora and in lactobacilli to ca. 5 to 7% (mean value) could be causally related to the subsequent diagnosis of decay. The expression of decay in the LCA subjects followed within 6 to 12 months the *S. mutans* overgrowth, whereas in the HCA subjects, decay was not detected until 18 to 30 months after this overgrowth occurred.

When the data were examined in a chronological fashion (Tables 8 and 9), about half the teeth which became decayed were already highly infected with *S. mutans*, i.e., 28 to 30% (mean value), and had a positive MSR at entry into the study. Because of the magnitude of this prior infection, it

TABLE 11. Mean proportion of lactobaccilli in teeth

Time (mo) before diagnosis	Mean proportion (%) of lactobacilli in teeth[a]		
	D	F	CF
0	8.3[b] (10)		1.9[b] (45)
6	7.5[b] (10)	3.2 (15)	1.1[b] (44)
12	0.5 (8)	1.5 (14)	3.0 (39)
18	0.4 (6)	.7 (11)	1.2 (41)

[a] Mean proportions are measured as a function of time before the diagnosis of decay in teeth that erupted during the period of observation. Values within parentheses are the number of teeth.
[b] Values with the same superscript are significantly different ($P < 0.05$) by the Scheffe test.

was not possible to show an incremental increase in *S. mutans* when these teeth were diagnosed as decayed 6 to 18 months later. The remaining teeth which initially had lower proportions of *S. mutans* and a negative MSR eventually showed abrupt increases in percent *S. mutans* and a positive MSR which preceded the diagnosis of decay by ca. 6 to 24 months.

Teeth that erupted during the course of this study showed the best temporal relationship between *S. mutans* overgrowth and the diagnosis of caries (Tables 10 and 11). When *S. mutans* proportions increased to more than 20%, decay was diagnosed within 6 months. Again no clear temporal relationship between an increase in percent *S. mutans* and the placement of a dental filling was observed.

However, it is apparent that overgrowth of *S. mutans* was not the exclusive determinant of decay because of the delayed expression of decay in some teeth after this overgrowth occurred and because of the absence of decay on certain teeth with moderately high levels of *S. mutans* in the caries-inactive or CF individuals. This suggested that other segments of the plaque flora might alone or in concert with *S. mutans* provide the actual odontopathic complex. Such changes were sought among the monitored species.

The lactobacilli increased 6 to 12 months before the diagnosis of decay in both the LCA and HCA subjects (Table 3). They also increased significantly 6 months before the diagnosis of caries in the teeth that erupted during the course of this investigation (Table 11). They were the only organisms of those monitored that tended to increase in the teeth that were destined to be F. Thus their credentials as a cariogen would seem to rival that of *S. mutans* except when their low proportions in the plaque are taken into account. The medium proportions of *S. mutans* on the D teeth in the LCA and HCA subjects were 19.2 and 17.0%, respectively, whereas the corresponding values for the lactobacilli were 0.3 and 0.8% (Tables 2 and 3). On many D teeth lactobacilli could not be detected. However, on those teeth where lactobacilli proportions were elevated, decay was almost inevitable.

In recent years an increase in plaque lactobacilli at the time of caries diagnosis has been observed (1, 11, 21) and has been interpreted as being secondary to the appearance of the clinical lesion (11, 19). However, Bowden and his colleagues found a relationship between lactobacilli and the risk of an incipient lesion progressing to the point where it needs to be replaced by a dental restoration (G. H. W. Bowden, *in* B. Guggenheim (ed.), *Cariology Today*, in press). In their study, the proportions of lactobacilli were also quite low relative to *S. mutans*. The relationship between lactobacilli and the placement of a dental restoration is observed in our study as teeth in the HCA subjects which received a dental filling by a private dentist tended to have high proportions of lactobacilli (Table 3).

The other monitored species did not seem to be cariogens. The high values of veillonellae in the carious teeth at the time of diagnosis was more a reflection of a decline in their proportions in the teeth that remained CF (Table 5). The veillonellae can use lactic acid as a nutrient source (24). Thus, their persistence in fissures destined to become D might reflect the continued availability of lactic acid in these plaques due to the higher proportions of lactic acid-producing organisms such as *S. mutans*. The actinomyces-like organism showed no remarkable changes in the plaque over time (Table 6). Its significantly higher proportions in the CF teeth in the CF subjects, which was not large in absolute terms, might represent a relative increase, due to the fact that the proportions of *S. mutans*, lactobacilli, and *veillonellae* were low in these plaques. *S. sanguis*, normally the predominant streptococcal species in young plaques (19), was, via the MSR, negatively associated with caries.

There were a few teeth on which the *S. mutans* proportions were high and caries did not occur. In this situation the high proportions of *S. mutans* suggest high-sucrose exposure which enabled *S. mutans* to establish dominance. The absence of caries in this situation may reflect that *S. mutans* is located mainly or exclusively at the orifice of the fissure, where the sample was taken, and was not present or metabolically active in the depths of the fissure where caries can initiate. Or it could indicate that a cocariogen, such as lactobacilli which might be necessary for the incipient lesion to progress (Bowden, in press) (Tables 10 and 11), was absent from these plaques. It could also reflect a resistant tooth surface, the presence of bacteria such as veillonellae which can consume acid, the presence of thin plaques that can be neutralized by salivary buffers so as to maintain the plaque pH above the critical pH for enamel demineralization (28), and other phenomenon yet to be described.

Collectively these bacteriological data show that plaques associated with the development of fissure decay usually harbor *S. mutans* in proportions that average ca. 20 to 25% of the cultivable flora before the detection of decay. These proportions were higher than those of *S. sanguis*, lactobacilli, veillonellae, and the unidentified actinomyces-like organism and probably were higher than that of any other single acidogenic or aciduric organism, or both, that was present in the plaque but was not monitored. This then basically would constitute the evidence that *S. mutans* is an important human odontopathogen. However, these findings do not rule out the possibility that other constellations of acidogenic-aciduric plaque organisms can comprise a cariogenic challenge to the tooth surface.

The present observations and others (1, 4, 5, 10, 11, 26, 30) provide sufficient documentation that a measurable amount of human dental decay is due to a treatable *S. mutans* infection (19). Accordingly, a strategy for caries control would be the early detection of such an infection and prompt antimicrobial or dietary treatment of such (1, 13, 15, 31; W. J. Loesche, *in* B. Guggenheim (ed.), *Cariology Today*, in press). The permanent teeth in the present group of children were infected with *S. mutans* shortly after their eruption. This indicates that tactics to prevent or to control an *S. mutans* infection should begin with the primary teeth as these teeth are the most likely sources of infection for the permanent teeth. When dentists were able to provide preventive measures to preschool children attending a pediatric clinic, they were able to obtain, over a 5-year period, about a 50% reduction in caries incidence (9). It may be that these preventive procedures should also be performed on the mother or principle caretaker of the child, as these individuals serve as the primary source of the *S. mutans* infections for the child (14, 29). Treatment of mothers, so as to reduce salivary *S. mutans* levels, resulted in a reduced incidence of *S. mutans* colonization in the child (15) and, subsequently, to a reduced caries incidence in the first years of life (B. Kohler, Arch. Oral Biol., in press).

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