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THE SALIVARY CONCENTRATION OF *STREPTOCOCCI MUTANS* AND *STREPTOCOCCI SANGUIS* AND THEIR COLONIZATION OF ARTIFICIAL TOOTH FISSURES IN MAN

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Summary—Artificial fissures were inserted in 3 subjects for 1–21 days. At a salivary concentration of 10^3 colony-forming units (CFU) *Strep. mutans*/ml, fissures were colonized by this organism. The proportional distribution of *Strep. mutans* increased with time and comprised up to 6 per cent of the total number of organisms in the fissure at day 21. The total number of organisms in the fissure did not increase with time after 1 day. The salivary concentration of *Strep. sanguis* was about 10^6 CFU/ml in all subjects. The proportional distribution of this organism in the fissure varied both between different individuals and with time in the same individual. When the artificial fissures were inserted during a regimen which reduced the salivary concentrations of *Strep. mutans*, the organism did not colonize the artificial fissures, even though the salivary concentration of *Strep. mutans* after cessation of the regimen increased to levels generally associated with colonization. If the fissures had been inserted 2 weeks before the *Strep. mutans* reducing regimen was started, the reduction of the number of *Strep. mutans* in the saliva to levels generally not associated with colonization did not influence the proportional distribution of *Strep. mutans* in the artificial fissure. These observations indicate that the initial inoculum is a main determinant for the colonization of the artificial fissures by *Strep. mutans*.

INTRODUCTION

Strep. mutans is significantly increased in fissure plaque associated with dental decay (Ikeda, Sandham, and Bradley, 1973; Loesche *et al.*, 1975). The microbial flora of fissures is difficult to assess completely because of the inability of sampling devices to penetrate the narrow orifice of the fissure. Fissure models have been devised which permit the quantitative recovery of bacteria from a fissure (Theilade, Larson and Karring, 1973). These models consist of Mylar fissures placed in gold inlays (Loe, Karring and Theilade, 1973) or natural fissures of unerupted third molars (Folke, Sveen and Thott, 1973; Theilade *et al.*, 1974) secured by various means. A Gram-positive coccal flora dominated in both model fissures (Theilade *et al.*, 1973, 1974; Thott, Folke and Sveen, 1974). *Strep. sanguis* was uniformly isolated and appeared to be the predominant organism. *Strep. mutans* was infrequently found in fissures placed in Danish subjects (Theilade *et al.*, 1973, 1974) but was found in all subjects and in most fissures of American subjects (Thott *et al.*, 1974) The salivary levels of *Strep. mutans* and *Strep. sanguis* appear to be important determinants in the colonization of smooth surfaces (van Houte and Green, 1974).

MATERIAL AND METHODS

Three adult subjects participated. The decayed, missing, filled surfaces (DMFS) scores were 38 in male subject *G*, 52 in male subject *T* and 78 in female *S*. The subjects had no missing teeth and no active carious lesions. Subjects *G* and *T* brushed and flossed their teeth daily, whereas *S* brushed but did not floss and used toothpicks occasionally. Subject *S* ate sweets between meals, whereas *T* and *G* did not.

The three subjects had an occlusal surface on a molar tooth prepared to receive a gold inlay containing a Mylar bag with the shape and dimensions of a natural fissure. This Mylar fissure and gold inlay, hereafter referred to as an artificial fissure, were autoclaved and secured with gutta percha in a maxillary first molar in *G*, and in mandibular first molars in *T* and *S*. The artificial fissures were always inserted in the mornings. After removal from the mouth, the Mylar bags were separated from the gold inlay using sterile instruments.

Unstimulated saliva was collected twice daily, morning and afternoon in the 1, 2 and 5 day experiments. In the longer experiments, saliva was sampled once daily, in the morning, with the exception of weekends when no samples were collected. Saliva was sampled immediately before the insertion and removal of the artificial fissures. One ml of saliva was added to 9 ml of reduced transport fluid (RTF)(Syed and Loesche, 1972). The inlay containing the artificial fissure was removed at various time intervals and the content of the artificial fissure placed into 10 ml of RTF. The samples were dispersed by sonification for 5 s (Branson W 185 D, N.Y.), serially diluted in RTF, and plated in duplicate over a 3 log dilution range on mitis-salivarius-bacitracin agar (MSB) (Gold, Jor-

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dan and van Houte, 1973) and MM10 sucrose agar (Loesche and Syed, 1973). The plates were incubated at 37°C in 85 per cent N_2, 10 per cent H_2 and 5 per cent CO_2 for 48 h. All samples were processed and placed in the anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) less than 30 min after collection. *Strep. mutans* was identified by colonial morphology on MSB agar and by mannitol fermentation of representative isolates. *Strep. sanguis* was identified as an adherent raised colony on MM10 sucrose agar (Loesche and Syed, 1973). The total colony-forming units count was obtained from MM10 sucrose agar plates.

Subjects *T* and *S* had relatively high levels of *Strep. mutans* in their saliva and the artificial fissures placed in their mouths were readily colonized by *Strep. mutans*. Experiments were performed to lower their salivary *Strep. mutans* levels to observe what effect this would have on *Strep. mutans* colonization of the artificial fissures. A mouthwash solution containing 5 ml of 0.4 per cent stannous fluoride and 5 ml of 0.02 per cent acidulated phosphate fluoride (Iradicav®, Janar Co., Grand Rapids, Mi.) was used daily after brushing and flossing the teeth in the evening. Ingestion of sucrose between meals was avoided. This regimen was performed for 14 days prior to the insertion of the artificial fissures. Additional experiments were performed with subject *S*. In two experiments, the artificial fissures were inserted while subject *S* was on the *Strep. mutans* reducing regimen and in two experiments the artificial fissures were *in situ* for 14 days prior to the introduction of the *Strep. mutans* reducing regimen.

RESULTS

The artificial fissures contained, after one day, a large number of microorganisms (Table 1). The total number did not change appreciably with time but the proportional distribution of *Strep. mutans* and *Strep. sanguis* varied considerably. *Strep. mutans* colonized the artificial fissures in two of the test subjects on each occasion, but in subject *G* this organism was only detected on two occasions i.e. 9 and 12 days after insertion of the fissures.

Strep. sanguis colonized the artificial fissures in all 3 persons on all occasions. There was, however, considerable inter- and intra-individual variation with time of the proportional distribution of this organism. For example, in subject *G*, *Strep. sanguis* accounted for about 4–6 per cent of the total CFU on days 1 and 2 and increased to 21 per cent on days 12 and 21. In subject *S*, on the other hand, *Strep. sanguis* was prominent on days 1 and 2 but decreased with time. In this subject, *Strep. mutans* was present in low proportions i.e. < 1 per cent on days 1 to 7 but increased to 6 per cent of the cultivable flora by day 21. Subject *T* showed about the same pattern as subject *S* with regard to *Strep. mutans*, but in *T* *Strep. sanguis* accounted for 10–21 per cent of the total CFU at all times.

The salivary concentrations of *Strep. sanguis* were about 10^6 CFU/ml in all subjects (Table 2), but the concentration of *Strep. mutans* varied considerably. In *G* in whom the artificial fissures were colonized by *Strep. mutans* on two occasions, this organism constituted a minor part of the cultivable flora i.e. 0.01–0.02 per cent, and the average salivary number was about 10^2/ml (Table 3). Subjects *T* and *S* had an average value of about 10^4 and 10^6 CFU *Strep. mutans* per ml saliva respectively, and their artificial fissures were colonized by *Strep. mutans* on all occasions. These data suggest that colonization of the artificial fissures by *Strep. mutans* takes place within one day, provided that the salivary concentration of this organism is above a certain level.

To test this hypothesis, the artificial fissures were re-inserted multiple times into the 3 subjects and left in place for 1, 2 or 5 days (Table 4). In *G* on 15 separate occasions, the artificial fissures were not colonized by *Strep. mutans*, but in *T* and *S* they were readily colonized by this organism at all trials. Subject *G* had an average of 2.5×10^2 CFU/*Strep. mutans* per ml saliva and only 7 out of 95 separate cultures showed a count $> 10^3$ CFU/ml. The average salivary *Strep. mutans* *in T* was 1.0×10^4 and in *S* was 0.4×10^6 CFU/ml (Table 4). The lowest average salivary *Strep. mutans* count in *T* in which the artificial fissures became colonized by this organism was 1.0×10^3 CFU/ml, whereas, in subject *S*, this low value was about 3.2×10^4 CFU/ml.

A *Strep. mutans* reducing regimen (SMRR), consisting of a combination of daily brushing and flossing

Table 1. *Strep. mutans* and *Strep. sanguis* as percentage of total colony-forming units (CFU) in the artificial fissure (AF) after 1, 2, 5, 7, 9, 12 and 21 days colonization in subjects *G*, *T* and *S*

	G			*T*			*S*		
Subject Time	*Strep. mutans* %	*Strep. sanguis* %	Total CFU*	*Strep. mutans* %	*Strep. sanguis* %	Total CFU	*Strep. mutans* %	*Strep. sanguis* %	Total CFU
1 day	0	5.6	4.5×10^7	0.03	10.5	0.7×10^7	0.02	17.6	0.5×10^7
2	0	3.9	9.7	0.1	15.2	0.4	0.1	24.0	0.8
5	0	10.9	3.5	0.03	12.2	3.3	0.02	4.2	5.3
7	0	18.5	3.4	0.02	19.5	5.1	0.9	6.3	2.0
9	0.02	15.6	4.8	1.1	14.2	2.5	1.4	5.3	1.3
12	0.01	20.8	1.5	1.3	20.8	2.1	3.8	4.6	0.8
21	0	26.9	1.1	4.4	19.2	0.9	6.3	1.1	1.2

* Total viable count on MM10 sucrose agar incubated anaerobically for 48 h.

Table 2. Concentration of *Strep. sanguis* in saliva during periods of colonization of the artificial fissures in subjects *G*, *T* and *S*

Subject / Time	*G*	$\bar{x}$* range	*T*	$\bar{x}$* range	*S*	$\bar{x}$* range
1 day	*n*† = 3	0.5 (0.3–0.8)	*n* = 3	5.0 (3.0–8.0)	*n* = 3	1.6 (1.3–2.0)
2	*n* = 5	2.5 (0.4–6.4)	*n* = 5	0.6 (0.5–1.0)	*n* = 5	2.5 (1.0–4.0)
5	*n* = 11	2.0 (0.4–4.0)	*n* = 11	3.0 (0.1–6.4)	*n* = 11	5.1 (0.2–10.0)
7	*n* = 5	0.6 (0.6–0.6)	*n* = 5	1.6 (0.8–3.0)	*n* = 7	2.0 (1.3–6.4)
9	*n* = 8	0.8 (0.5–1.3)	*n* = 8	1.3 (0.8–6.4)	*n* = 8	1.6 (1.0–6.4)
12	*n* = 10	1.6 (0.4–4.0)	*n* = 5	0.5 (0.3–5.1)	*n* = 11	1.3 (0.5–4.0)
21	*n* = 15	1.3 (0.4–4.0)	*n* = 11	0.6 (0.3–13.1)	*n* = 16	1.6 (0.6–6.4)
Average	*n* = 57	1.3 (0.3–4.0)	*n* = 48	1.3 (0.1–13.1)	*n* = 61	2.0 (0.2–10.0)

$\bar{x}$* = mean number of *Strep. sanguis* per ml of saliva × 10^6.
n† = number saliva samples collected at each time trial.

of the teeth, daily fluoride rinses and between-meal sucrose restriction was administered for two weeks prior to a series of experiments in which the artificial fissure was re-inserted multiple times in subjects *T* and *S*. This SMRR lowered the salivary levels of *Strep. mutans* to an average of 0.8 × 10^3 CFU/ml in subject *T* during the several weeks in which the experiments were performed (Table 5). Only 4 out of 10 artificial fissures inserted in *T* became colonized by *Strep. mutans*. When colonization of the fissures occurred, the average salivary *Strep. mutans* level ranged from 0.3 to 4.0 × 10^3 CFU/ml. The artificial fissures did not become colonized by *Strep. mutans* on 6 occasions when similar levels of this organism were present in the saliva. The detection of *Strep. mutans* in the artificial fissure in subject *T* appeared to be a function of time, as no artificial fissures were colonized by *Strep. mutans* after 1 day *in vivo*, 2 of 4 were colonized by *Strep. mutans* after 2 days, and 2 of 3 were colonized by *Strep. mutans* after 5 days (Table 5). In *S*, the reduction of salivary *Strep. mutans* levels did not prevent colonization of the artificial fissure by this organism at any time period.

The experimental protocol was changed and the artificial fissure was inserted during a *Strep. mutans* reducing regimen in subject *S*. In one experiment (Fig. 1a), the artificial fissure was inserted at zero time and removed after 14 days. The *Strep. mutans* reducing regimen was administered for the first 7 days, during which time the salivary levels of *Strep. mutans* ranged from 1.6 to 6.5 × 10^3 CFU/ml. After cessation of the

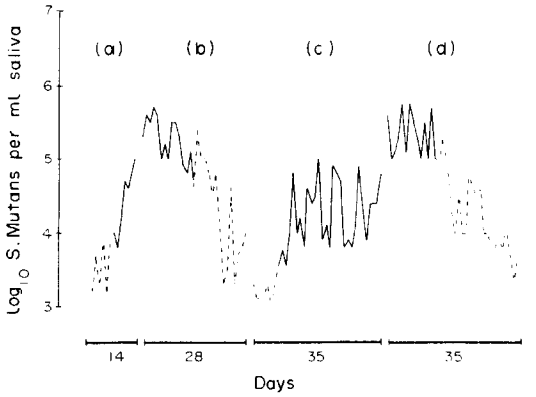

Fig. 1. Variations in salivary *Strep. mutans* concentration in subject *S* during different experimental conditions of the SMRR. ·–·–·– during the SMRR; —— in the absence of the SMRR. The four experiments are designated as a, b, c, and d.

Table 3. Concentration of *Strep. mutans* in saliva during periods of colonization of the artificial fissures (AFs) in subjects *G*, *T* and *S*

Subject / Time	*G* counts × 10^2 $\bar{x}$* range		*T* counts × 10^4 $\bar{x}$* range		*S* counts × 10^6 $\bar{x}$* range	
1 day	*n*‡ = 3	0.5(ND†–1.5)	*n* = 3	0.8 (0.2–2.0)	*n* = 3	0.4 (0.2–0.6)
2	*n* = 5	2.0 (ND–7.0)	*n* = 5	0.6 (0.3–1.6)	*n* = 5	1.0 (0.2–1.6)
5	*n* = 11	0.2 (ND–2.0)	*n* = 11	3.2 (0.5–13.0)	*n* = 11	1.0 (0.1–3.2)
7	*n* = 5	20.0 (7.0–70.0)	*n* = 5	3.2 (1.0–8.0)	*n* = 7	1.2 (0.3–4.0)
9	*n* = 8	26.0 (10.0–70.0)	*n* = 8	1.6 (0.1–6.4)	*n* = 8	0.6 (0.2–1.2)
12	*n* = 10	26.0 (1.0–40.0)	*n* = 5	2.0 (0.5–6.4)	*n* = 11	1.2 (0.6–8.0)
21	*n* = 15	1.6 (ND–10.0)	*n* = 11	0.8 (0.1–1.6)	*n* = 16	1.0 (0.1–5.0)
Average	*n* = 57	2.5 (ND–70.0)	*n* = 48	1.6 (0.1–13.0)	*n* = 61	1.0 (0.1–8.0)

$\bar{x}$* = mean number of *Strep. mutans* per ml saliva.
ND† = not detectable.
n‡ = number saliva samples collected during each time trial.

Table 4. Concentrations of *Strep. mutans* in saliva during 1, 2 and 5 days of colonization of the artificial fissures (AFs) in subjects *G*, *T* and *S*

Subject	*G*					
Time	No. experiments	*n**	*Strep. mutans* × 10^2 per ml saliva $\bar{x}$† range	*Strep. mutans* in AF	No. experiments	n
1 day	5	15	1.7 (ND–13.0)‡	ND	4	12
2 days	5	25	3.0 (ND–21.0)	ND	4	20
5 days	5	55	2.9 (ND–21.0)	ND	4	44
Average	15	95	2.5 (ND–21.0)	0/15§	12	76

*n** = number of saliva samples collected during each time trial.
$\bar{x}$† = mean number of *Strep. mutans* per ml saliva.
ND‡ = not detectable.

Strep. mutans reducing regimen, the salivary levels of *Strep. mutans* increased within the first few days to about 10^5 CFU/ml. When removed after 14 days, the artificial fissure contained the expected total number of microorganisms, including a large proportion of *Strep. sanguis*, but no detectable *Strep. mutans* (Table 6).

The artificial fissure was re-inserted and left *in vivo* for 28 days. The salivary *Strep. mutans* levels ranged from about 0.9×10^5 to 5×10^5 CFU/ml during the first 14 days (Fig. 1b). The *Strep. mutans* reducing regimen was initiated on day 15 and continued to day 28. The salivary *Strep. mutans* levels decreased by day 21 to about 2×10^3 organisms per ml. The artificial fissures were removed at day 28 and contained about 10 per cent *Strep. mutans* and 14 per cent *Strep. sanguis* (Table 6).

Both experimental variations of the *Strep. mutans* reducing regimen were repeated. The fissure was re-inserted while subject *S* was on the *Strep. mutans* reducing regimen (Fig. 1c). The regimen was continued for 1 week during which the salivary levels of *Strep. mutans* were about 10^3 organisms per ml. The regimen was stopped at day 8 and the artificial fissure was left *in vivo* until day 35. The levels of *Strep. mutans* increased immediately and ranged from 5×10^3 to about 1×10^5 organisms per ml of saliva during this 28 day period. When the artificial fissures was cultured at day 35, no *Strep. mutans* were detectable even though about 1×10^7 CFU were present in the fissure (Table 6).

The fissure was next inserted while subject *S* was off the *Strep. mutans* reducing regimen and had salivary *Strep. mutans* levels of about 1×10^5 to 5×10^5 organisms per ml of saliva (Fig. 1d). The artificial fissure was exposed to these salivary levels for 2 weeks, after which the *Strep. mutans* reducing regimen was initiated at day 15 and continued until day 35. The salivary *Strep. mutans* levels declined about 50 fold during this 21-day period, but the artificial fissure levels of *Strep. mutans* appeared to be unaffected (Table 6). This could be demonstrated by plotting the percentage of *Strep. mutans* in the artificial fissure against the number of days in which the fissure was present *in vivo* in subject *S*. The percentages of *Strep. mutans* in the artificial fissure at 9, 12 and 21 days in the absence of the SMRR (data taken from Table 1) and at 28 and 35 days in the presence of the SMRR

Table 5. Salivary concentrations of *Strep. mutans* during periods of colonization of the artificial fissures after 2 weeks of a *Strep. mutans* reducing regimen in subjects *T* and *S*

Subject	*T*			*S*		
Time		counts × 10^3 $\bar{x}$* range	*Strep. mutans* in AF		counts × 10^4 $\bar{x}$* range	*Strep. mutans* in AF
1 day	*n*‡ = 3	0.4 (0.2–0.6)	ND†	*n* = 3	2.6 (1.0–2.8)	+
1	*n* = 3	0.1 (0.1–0.1)	ND	*n* = 3	2.8 (1.6–4.0)	+
1	*n* = 3	0.3 (0.2–0.3)	ND			
2 days	*n* = 5	0.6 (0.1–1.3)	ND	*n* = 5	4.0 (1.0–13.0)	+
2	*n* = 5	0.6 (0.1–1.6)	+	= 5	4.0 (0.6–8.0)	+
2	*n* = 5	4.0 (1.0–10.0)	ND			
2	*n* = 5	0.3 (0.2–0.6)	+			
5 days	*n* = 11	1.0 (0.1–4.0)	ND	*n* = 11	4.0 (1.0–13.0)	+
5	*n* = 11	3.0 (0.6–6.0)	+	*n* = 11	5.0 (0.2–13.0)	+
5	*n* = 11	4.0 (0.8–13.0)	+			
Average	*n* = 62	0.8 (0.1–13.0)	4/10+	*n* = 38	3.2 (0.2–13.0)	6/6+

* $\bar{x}$ = mean number of *Strep. mutans* per ml saliva.
† ND = not detectable.
‡ *n* = number saliva samples collected during each time trial.

Table 4. Continued

T *Strep. mutans* × 10^4 per ml saliva x̄† range	*Strep. mutans* in AF	No. experiments	n	*S* *Strep. mutans* × 10^6 per ml saliva x̄† range	*Strep. mutans* in AF
0.8 (0.02–5.1)	+	3	9	0.2 (0.006–0.9)	+
1.1 (0.01–10.0)	+	3	15	0.4 (0.004–1.6)	+
1.0 (0.01–12.0)	+	3	33	0.4 (0.01–3.2)	+
1.0 (0.01–12.0)	12/12§	9	57	0.4 (0.004–3.2)	9/9§

§ number of times *Strep. mutans* was detected in AF divided by total number of experiments.

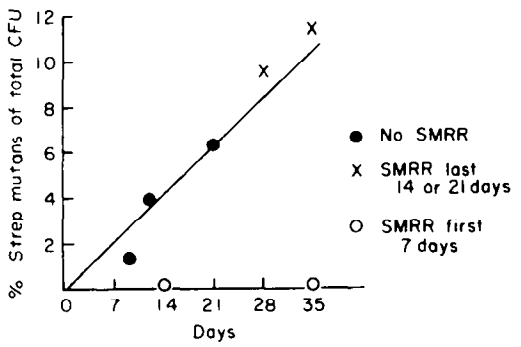

Fig. 2. The proportions of *Strep. mutans* in the artificial fissure in subject *S* during different time periods and experimental conditions.

fell on the same line (Fig. 2). The two points obtained when the artificial fissure was inserted at the same time that the SMRR began are not on this line.

DISCUSSION

The artificial fissures were colonized within 1 day by a large number of microorganisms. The total number did not change appreciably in the fissures left *in situ* for 2–3 weeks, indicating that the majority of space is quickly filled with bacteria and that the microorganisms remain viable in the fissure for a long period.

The microorganisms must have been introduced in the artificial fissure via the saliva or translocated via contact from adjacent tooth surfaces as suggested by Löe *et al.* (1973). We found that the salivary levels of *Strep. sanguis* and *Strep. mutans* could be associated with their presence in artificial fissures. *Strep. sanguis* averaged about 10^6 CFU/ml of saliva. This organism colonized the fissures in the 3 subjects on all occasions and comprised from 6 to 18 per cent of the cultivable flora after 1 day. *Strep. mutans* colonized the artificial fissures on all occasions in the two subjects in whom the salivary counts of *Strep. mutans* were about 10^4 CFU/ml or higher. In the third subject, in whom the salivary number of *Strep. mutans* usually averaged about 10^2 CFU/ml, *Strep. mutans* was detected in the artificial fissure only when the fissures were left in the mouth for a long period. This observation suggests either that the initial inoculation of the artificial fissures contained so few *Strep. mutans* that a long time was necessary for *Strep. mutans* to attain numbers sufficient for detection, or that *Strep. mutans* is a late arrival depending upon time and chance eventually to come in contact with the fissure. A third possibility is that certain conditions in the fissure brought about by other organisms eventually favours later colonization by *Strep. mutans*.

Our observations indicate that the salivary concentration of *Strep. mutans*, at which colonization of the artificial fissure occurs within the first few days, must be somewhere between 10^2–10^4 CFU/ml. After the salivary concentration of *Strep. mutans* had been reduced by oral hygiene, fluoride and dietary regimens to 10^3 CFU/ml in subject *T*, the artificial fissures were not colonized by this organism at day 1. However, they were colonized by *Strep. mutans* at 2 and 5 days in 4 of 7 trials. This would place the colonization threshold somewhat below 10^3 CFU/ml in these subjects. This value is lower than the salivary *Strep. mutans* level of 4.5 × 10^4 CFU/ml calculated

Table 6. Colonization of the AF by *Strep. mutans* and *Strep. sanguis* during the *Strep. mutans* reducing regimen (SMRR) in subject *S*

AF *in vivo*	*Strep. mutans* (%)	*Strep. sanguis* (%)	Total viable count × 10^7
	SMRR first 7 days *in vivo*		
14 days	0	8.5	3.7
35	0	6.9	0.9
	SMRR last 14 or 21 days *in vivo*		
28 days	9.6	13.5	3.3
35	11.5	12.6	1.8

by van Houte and Greene (1974) to be necessary for *Strep. mutans* to colonize a non-retentive smooth surface within 2 to 3 h. That this value should be lower in the fissure is not surprising, as the fissure provides a stagnant space less accessible to the salivary flow dynamics and masticatory abrasive effects that occur on the smooth surfaces. Our findings suggest that fissures would be the first tooth surface areas colonized by *Strep. mutans*, which is in agreement with clinical data (Ikeda and Sandham, 1971).

The values of 10^3 CFU/ml for colonization of the artificial fissure and 4.5×10^4 CFU/ml for colonization of smooth surfaces are based upon studies in which the saliva was homogenized by either a sonifier, as in our investigation, or a Vortex mixer (van Houte and Green, 1974). Such evenly dispersed salivary concentrations of *Strep. mutans* would not be found *in vivo*, where one would expect certain small volumes of saliva to be high in *Strep. mutans* and others to be low or devoid of this organism. Thus the *Strep. mutans* colonization threshold in saliva as determined by experimental data is an average and not an absolute value.

When *Strep. mutans* was found in the artificial fissures in subject *G*, the *Strep. mutans* to *Strep. sanguis* ratio in these fissures was less than 0.001, reflecting a noncariogenic pattern (Loesche *et al.*, 1975). This subject had the lowest salivary concentration of *Strep. mutans* and the lowest DMFS score. Conversely, in subject *S*, the *Strep. mutans* to *Strep. sanguis* ratio was below 0.01 at days 1, 2 and 5 and then increased, so that by day 21 the value was 5.7. This reflects a cariogenic pattern and this subject had the highest salivary concentration of *Strep. mutans* and the highest DMFS score. Subject *T* had an intermediate *Strep. mutans* to *Strep. sanguis* ratio, an intermediate salivary *Strep. mutans* concentration and an intermediate DMFS score.

The increase in proportion of *Strep. mutans* with time in a fissure noted by us and by Thott *et al.*, 1974 indicates that *Strep. mutans* possesses some advantage in the crowded confines of a fissure. The organisms in the initial inoculum may be the main, if not exclusive, determinants of the fissure flora at any time. If so, the eventual appearance of *Strep. mutans* in the fissure seen in subjects *G* and *T* after the *Strep. mutans* reducing regimen, would be the result of a small initial inoculum of *Strep. mutans* increasing with time to detectable levels, rather than a later colonization of the fissure by this organism. The studies performed on subject *S*. by varying the time sequence in which the *Strep. mutans* regimen was given and the artificial fissure was inserted, also support the suggestion that the initial inoculum determines the subsequent flora of the fissure. If the fissure was inserted during a period of SMRR, no colonization of the fissure by *Strep. mutans* occurred, even when the fissure was left *in situ* for additional 1 to 4 week period.

The failure of *Strep. mutans* to colonize the artificial fissure during the week of the SMRR could be attributed to the reduction of salivary *Strep. mutans* levels to values approaching the colonization threshold level of 10^3 CFU/ml/min. (Fig. 1a and c) and/or to the accumulation of inhibitory levels of fluoride in the fissure and/or to the reduction of sucrose intake. If residual fluoride was present in the artificial fissure, it did not affect the total viable count and did not inhibit the *Strep. sanguis* colonization. However, a more specific effect on *Strep. mutans* adhesion is possible.

After the cessation of the SMRR the artificial fissures did not become colonized by *Strep. mutans* although the salivary concentration of this organism increased to values in excess of the colonization threshold. A probable explanation is that all the available space in the artificial fissures was already occupied by other microorganisms and no ecological niche was left for *Strep. mutans*. The experiments in which the artificial fissures were left *in situ* for two weeks prior to the introduction of the SMRR showed that the proportional increase in *Strep. mutans* was the same as in those experiments in which time was the only variable (Fig. 2). These data suggest that the microflora in the artificial fissure is a relatively closed system. Colonization by new organisms like *Strep. mutans* seems to be rare after the fissures have been occupied by a large number of organisms, i.e. within 1 day, and the confined environment favours the growth of some species such as *Strep. mutans*.

These observations suggest that antimicrobial efforts directed specifically against *Strep. mutans* about the time fissure surfaces enter the mouth may prevent this organism from colonizing these highly caries-susceptible surfaces. They also imply that antimicrobial therapy given after a fissure is colonized by *Strep. mutans* may have little if any effect on the fissure levels of *Strep. mutans*.

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