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Relationship Between the Concentration of Bacteria in Saliva and the Colonization of Teeth in Humans

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The relationship between the salivary concentration of bacteria and their number that can be recovered from tooth surfaces has been studied in 12 human subjects. The mean salivary concentration of naturally occurring *Steptococcus mutans* and lactobacilli, determined on selective media, was 3.7×10^5 and 3.8×10^5 colony-forming units (CFU) per ml, respectively. In subjects with salivary concentrations of *S. mutans* of about 10^4 CFU/ml or less or about 10^5 CFU/ml or less of lactobacilli, these organisms could not be isolated from cleaned teeth after 2 to 3 h of oral exposure. In experiments with streptomycin-labeled *S. sanguis* cells held in the mouth for 15 min, the minimal salivary concentration required for their recovery from the teeth was about 10^3 CFU/ml. Both *S. mutans* and lactobacilli were found to be highly localized on teeth. This evidence suggests that the concentrations of *S. mutans* and lactobacilli generally present in saliva are insufficient for the initiation of their firm attachment to relatively nonretentive tooth surfaces. The low efficiency of their intraoral spread, as suggested by their highly localized distribution on teeth, or of their transmission between subjects may be essentially due to the interrelated factors bacterial affinity and number of colony-forming units available for attachment.

Various studies indicate that human oral bacteria establish with different efficiency. For example, *Streptococcus salivarius* colonizes the mouth within days after birth (4), and *S. sanguis* colonizes directly after tooth eruption (5). On the other hand, the appearance of *S. mutans* is delayed even when the teeth, their apparent primary oral habitat, are present (5). In addition, various oral species that have distinct preferred habitats (2, 8, 16) differ in the extent to which they colonize. Thus, recent evidence suggests that *S. mutans* does not uniformly colonize the tooth surface but is highly localized (11, 12, 21; I. L. Shklair et al., abst. no. 71, Int. Ass. Dent. Res., 1972). It is especially found on tooth surface areas considered retentive such as fissures or approximal surfaces and in or near carious lesions. In one mouth it may be consistently isolated from certain tooth surfaces but not from others. Likewise, lactobacilli are predominantly associated with carious lesions that constitute their indispensable habitat (15, 20, 22). In contrast, *S. sanguis* is more ubiquitously present on the teeth and consequently comprises generally a higher proportion of dental plaque bacteria than both other organisms (2, 6, 25).

It may be inferred from these observations that in case of *S. mutans* and the lactobacilli, both implicated as etiological agents in dental caries (9, 17), successful intersubject transmission and spread from infected tooth surfaces to uninfected surfaces, especially the relatively nonretentive ones, occur only with great difficulty. In case of *S. sanguis*, on the other hand, both processes proceed with greater efficiency.

Previous investigations of bacterial attachment have demonstrated that oral bacteria, including the discussed species, differ widely in their affinity for oral surfaces (9). The present study deals with the relationship between the salivary concentration of *Streptococcus* sp. and lactobacilli and their numbers that can be recovered from teeth. The findings suggest that both bacterial affinity and bacterial numbers available for attachment constitute important, interrelated ecological determinants. A concept has been proposed that may explain the differences between various *Streptococcus* sp. and lactobacilli in their oral establishment.

MATERIALS AND METHODS

Experiments with labeled cells. Adult male and female employees at the Forsyth Dental Center with

varying caries experience served as subjects. *S. sanguis* strains H_7PR and $A_{12}R$ resistant to streptomycin, which have been previously described, were used (25). Both strains were cultivated aerobically in Trypticase soy broth (BBL) with 0.25% glucose for 16 h. The cells were washed twice with modified Ringer solution (24) and then suspended in the same solution. One-half milliliter of cell suspensions within the order of 10^7,10^6, 10^5, 10^4, 10^3, or 10^2 colony-forming units (CFU) per ml was introduced with a pipette in the mouth of the subjects and held there for 15 min. The subjects were instructed to distribute the cells throughout the mouth and to refrain from swallowing. At the end of the 15-min period, unstimulated saliva was collected. In addition, samples were obtained from the buccal and lingual surfaces of upper and lower anterior teeth (sampling area, 10 to 30 mm^2) covered with varying amounts of dental plaque by forceful swabbing with Calgiswabs (Colab Laboratories, Inc.). The gingival tooth surface area was avoided. The teeth were isolated with cotton rolls to prevent further salivary contamination prior to sampling but were not rinsed. The swabs were wetted by dipping in sterile vials with 1 ml of Ringer solution prior to use. During the sampling procedure, the swabs were slowly rotated in order to utilize the whole available surface area. The swabs, each used for a different surface, were replaced in the vial and permitted to stand for 1 min to dissolve the Calgiswab material and to effect a release of the attached bacteria. The vials were then shaken for 30 s on a Vortex mixer. Next, 0.3 ml of the undiluted suspensions as well as 0.1-ml samples of appropriate dilutions of the saliva samples in Ringer solution similarly mixed for 30 s were spread with bent glass rods on the surface of duplicate plates of mitis-salivarius agar (Difco) with 200 μg of streptomycin per ml for the enumeration of the labeled *S. sanguis*. The plates were incubated aerobically for 2 to 3 days prior to counting. The number of CFU of *S. sanguis* pipetted into the mouth was similarly determined immediately after their oral introduction. Prior to each experiment, the saliva and the tooth surfaces to be studied were examined to insure the absence of labeled organisms similar to those used in the experiments.

Experiments with naturally occurring cells. Adult male and female employees at the Forsyth Dental Center were screened for the presence of *S. mutans* and lactobacilli in their saliva. *S. mutans* was enumerated on a medium highly selective for this organism consisting of regular mitis-salivarius agar with 20% sucrose and 0.2 U of bacitracin per ml (10). This medium permits the detection of very low *S. mutans* numbers in oral samples. For lactobacilli, selective Rogosa SL agar (Difco) was employed. A number of subjects with varying salivary concentrations of both organisms (range, 10 to 10^6 CFU/ml) were selected for the study. During the experiments the subjects remained on their usual diet.

Lingual surfaces of upper anterior teeth were thoroughly cleaned to reduce the bacterial populations to a negligible level (24). The tooth surfaces were then exposed to the oral environment for 2 to 3 h. This period was chosen to allow the study of bacterial attachment and to exclude or minimize the influence of bacterial growth on the number of CFU recovered. Directly before oral exposure, the teeth were sampled with Calgiswabs as outlined previously to determine the efficacy of the prophylaxis. Also, about 1 ml of saliva was collected. At the end of the experimental period, during which the subjects resumed normal activities, another saliva sample was obtained, whereas the tooth surfaces to be studied (sampling area, 20 to 30 mm^2) were sampled with Calgiswabs as previously described. All swabs were placed in vials with 1.5 ml of Ringer solution. All samples were treated prior to culturing as outlined previously. Three-tenths milliliter of the suspensions of the tooth surface samples or 0.1 ml of appropriate dilutions of these samples and of the saliva samples were cultured on the media selective for *S. mutans* and the lactobacilli. The media were incubated in Brewer jars (BBL) filled with 80% N_2, 10% H_2, and 10% CO_2 for 3 days. For the purpose of comparison, samples were also cultured on mitis-salivarius agar plates (Difco) that were incubated aerobically for 2 days for the enumeration of *S. salivarius*. Identification of the organisms was based on their characteristic colonial morphology on the media employed.

Efficacy of swabbing technique. Appropriate numbers of cells of *S. sanguis* H_7PR were introduced in the mouth of three subjects and held there for 15 min. Four lingual surfaces of upper anterior teeth of each subject were sampled with Calgiswabs as described. This sampling procedure was immediately repeated once. Each of the two successive samples from each tooth surface was placed in separate tubes with 1 ml of Ringer solution and treated as usual prior to culturing on streptomycin-containing mitis-salivarius agar. In addition, to determine whether the bacteria were released from the Calgiswabs during standing and mixing, the swabs used for the first sampling after standing and 30 s of mixing were transferred to another series of tubes containing 1 ml of fresh, sterile Ringer solution. This procedure was repeated once.

The experiments demonstrated that nearly all bacteria that were recovered from the teeth were obtained during the first sampling; the organisms recovered during the second sampling constituted only from 0 to 12% of those obtained during the first sampling. In addition, most bacteria were found to be released from the swabs used for the first sampling. The numbers of organisms recovered from the swabs after the first transfer comprised only 1 to 9% of those recovered from the swabs used for the first sampling, whereas in all but two instances no bacteria could be recovered after the second transfer.

In other experiments, cleaned tooth fragments prepared from freshly extracted human teeth (24) were placed in the upper and lower anterior area of the mouth of some of the subjects for 2 h. After their oral exposure, they were removed from the mouth and sampled in a manner analogous to that used in vivo. Inspection with the scanning electron microscope of the areas on the fragments that had been swabbed and that had been left untouched showed that the in vivo sampling procedure was very effective in the

removal of the bacteria.

Distribution of S. mutans and lactobacilli on teeth. From each subject plaque samples were obtained with Calgiswabs and sterile scalers from a number of different tooth surfaces. Only a small area of about 5 mm^2 was sampled. All samples were treated as previously described. Appropriate dilutions were streaked on regular mitis-salivarius agar and the selective medium for the enumeration of *S. mutans*, whereas Rogosa SL agar and Trypticase soy agar with 5% sheep blood were employed for the enumeration of the lactobacilli. All plates were incubated in Brewer jars in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 for 3 days.

RESULTS

Experiments with labeled S. sanguis. Experiments with labeled *S. sanguis* showed that, as expected, the number of CFU that could be recovered from nonretentive tooth surfaces was dependant on the number of CFU introduced and their salivary concentration. Some typical results with strain H_7PR are shown in Table 1. The lower number of CFU in saliva at the end of the 15-min experimental period likely reflects dilution of the introduced cells by newly produced saliva and the adherence of cells to oral surfaces. There appeared to be a critical salivary concentration below which no cells could be recovered from the tooth surfaces (Table 1). With *S. sanguis* strain H_7PR as well as strain $A_{12}R$, this concentration was in the order of 10^3 CFU/ml.

Experiments with naturally occurring cells. The relationship between the salivary concentration of *S. mutans* and its recovery from tooth surfaces is shown in Table 2. The mean number of CFU recovered from the teeth as well as the number of tooth surfaces from which cells could be recovered decreased with decreasing salivary concentration. No cells were recovered in three subjects with salivary concentrations of about 10^3 to 10^4 CFU/ml. The mean number of CFU of *S. mutans* in saliva of the 12 subjects was 3.7×10^5 (Table 3). This number represents the mean of the averages of both saliva samples obtained at the start and end of the 2- to 3-h experimental period calculated for each subject separately. The salivary concentration of 7 of the 12 subjects was in the order of 10^4 CFU/ml or less. The mean salivary concentration of these 12 adult subjects was found to be comparable with a mean salivary concentration of *S. mutans* of 1.7×10^5/ml (range, 1.1×10^2 to 1×10^6) obtained for 25 children, 12 to 15 years of age, with varying caries experience. Fifteen of these children had a salivary concentration of about 10^4 CFU/ml or less. The mean of the salivary concentration of *S. mutans* that corresponded with the recovery of 1 CFU per tooth surface was 4.5×10^4/ml (Table 3). This concentration was first calculated for each of the nine subjects from whom *S. mutans* could be recovered from the teeth. The salivary concentration corresponding to 1 CFU in each subject was obtained by comparing the average of both salivary concentrations at the start and end of the experiment with the average number of CFU recovered per tooth surface.

The relationship between the salivary concentration of lactobacilli and *S. salivarius* and their recovery from the teeth is shown in Tables 4 and 5, respectively. Lactobacilli could not be isolated from the teeth in subjects with salivary concentrations up to 10^5 CFU/ml. Eight of the 12 subjects had salivary concentrations in the order of 10^5 CFU/ml or less. The mean number of lactobacilli in saliva was 3.8×10^5 (Table 3), and the mean of the salivary concentration corresponding to the recovery of 1 CFU per tooth surface was 3.0×10^5/ml. For *S. salivarius*, both means were 1.0×10^8 and 3.7×10^5, respectively.

The figures on the salivary concentration corresponding to the recovery of 1 CFU per tooth surface (Table 3) are also a measure of the relative affinity of the studied organisms for the teeth. In each subject, with only one exception, this salivary concentration was found to be lower for *S. mutans* than for *S. salivarius*, indicating a higher affinity of *S. mutans*. The mean ratio was 13.7 to 1 in favor of *S. mutans* ($0.01 < P < 0.05$; *t* test) with a range of 1-43

TABLE 1. *Relationship between the salivary concentration of streptomycin-labeled S. sanguis and its number recovered from the tooth surface*

Subject	No. of CFU introduced	No. of CFU/ml of saliva	No. of CFU per tooth surface[a]	
			Mean	Range
A	2.3×10^7	2.7×10^6	TNTC[b]	
	8.1×10^5	2.2×10^5	68	20-170
	2.1×10^5	8.3×10^4	42	10-66
	3.3×10^4	10^4	13	2-28
	1.9×10^4	2.1×10^3	1	ND-5
	1.9×10^3	3.0×10^2	ND[b]	
B	2.1×10^7	3.8×10^6	TNTC	
	9.2×10^5	2.5×10^5	198	13-383
	1.5×10^5	4.0×10^4	43	23-66
	9.7×10^3	1.8×10^3	6	2-17
	1.5×10^4	2.3×10^3	4	3-7
	1.8×10^3	3.0×10^2	<1	ND-3

[a] Mean of 6 to 10 tooth surfaces.

[b] TNTC, Too numerous to count on crowded plates. ND, Not detected.

TABLE 2. *Relationship between the salivary concentration of naturally occurring S. mutans and its number recovered from the tooth surface*

No. of subjects	No. of tooth surfaces	No. of CFU/ml of saliva (range)		No. of tooth surfaces positive	No. of CFU per tooth surface	
		Start	End		Mean	Range
3	9	6×10^5 to 3.3×10^6	3.5×10^5 to 1.2×10^6	7	31	ND[a]-145
6	20	4.5×10^4 to 3.6×10^5	1.9×10^4 to 8.5×10^4	10	10	ND-95
3	12	5×10^3 to 1.5×10^4	6.8×10^2 to 10^4	0	ND	

[a] ND, Not detected.

TABLE 3. *Mean salivary concentration and number of CFU per milliliter of saliva corresponding to 1 CFU per tooth surface*

Organism	No. of CFU/ml of saliva		No. of CFU/ml of saliva corresponding to 1 CFU per tooth surface[a]	
	Mean	Range	Mean	Range
S. mutans	3.7×10^5	2.8×10^3 to 2.3×10^6	4.5×10^4	2.4×10^3 to 2.0×10^5
Lactobacilli	3.8×10^5	1.5×10^1 to 3.0×10^6	3.0×10^5	4.4×10^4 to 10^6
S. salivarius	10^8	4.5×10^6 to 6.0×10^8	3.7×10^5	1.2×10^4 to 10^6

[a] Only including subjects from which organisms were isolated from the teeth.

TABLE 4. *Relationship between the salivary concentration of naturally occurring lactobacilli and their number recovered from the tooth surface*

No. of subjects	No. of tooth surfaces	No. of CFU/ml of saliva (range)		No. of tooth surfaces positive	No. of CFU per tooth surface	
		Start	End		Mean	Range
4	18	1.2×10^5 to 4.6×10^6	1.5×10^5 to 1.4×10^6	7	19	5-195
8	32	10 to 10^5	20 to 1.6×10^4	0	ND[a]	

[a] Not detected.

to 1 of the individual ratios. On the other hand, the affinity of *S. mutans* was found to be comparable with that of the lactobacilli, although these organisms did not differ in their affinity

TABLE 5. *Relationship between the salivary concentration of naturally occurring S. salivarius and its number recovered from the tooth surface*

No. of subjects	No. of tooth surfaces	No. of CFU/ml of saliva (range)		No. of CFU per tooth surface	
		Start	End	Mean	Range
10	42	4×10^6 to 5×10^8	2.8×10^6 to 7×10^8	492	0-3,150

from *S. salivarius*. Comparison of these figures was difficult due to the limited data available for the lactobacilli and the large variations between the individual data as indicated by the ranges given (Table 3).

Distribution of S. mutans and lactobacilli on teeth. Some examples of the proportional distribution of *S. mutans* and lactobacilli on different tooth surface areas in one mouth are shown in Table 6. The proportions of *S. mutans* as well as lactobacilli varied greatly from site to site, and over 1,000-fold differences in concentration were observed. Moreover, the proportions were with few exceptions very low. This was so in spite of the fact that the concentrations of *S. mutans* and lactobacilli in saliva, determined on different occasions during a period from about 1 month before sampling to 1 month thereafter, were in the order of 10^5 to 10^6 (Table 6) and, as such, at the high end of the concentration range of these organisms in human saliva.

DISCUSSION

In attempting to understand the regulation of the oral microbiota, it is helpful to consider the factors that may theoretically influence this process. These factors include (i) the affinity of

TABLE 6. *Proportions of S. mutans and lactobacilli on different tooth surfaces in the same human mouth*

Tooth surface site[a]	*S. mutans*[b]		Lactobacillus[c]	
	Subject A	Subject B	Subject C	Subject D
1	27.6	0.1	0.0005	0.01
2	0.2	1.0	0.0005	22.2
3	<0.2[d]	<0.2	0.00001	0.4
4	<0.4	<0.3	0.5	0.007
5	<1.4	4.5	0.03	0.1
6	<0.2	0.7	0.07	0.4
7	0.3	2.0	0.4	0.5
8	<0.05	<0.05	0.0006	0.003
9	<0.3	<0.1	0.0001	0.05
10	1.8	<0.4	0.001	<0.02
11	0.02	<0.2		
12	1.2	5.4		
13	0.07	<0.15		
14	0.05	<0.2		
No. of CFU/ml of saliva (range)	3×10^5 to 2×10^6	8×10^5 to 5×10^6	10^6 to 2.2×10^6	9×10^5 to 3×10^6

[a] *S. mutans:* samples obtained from buccal and lingual tooth surfaces; Lactobacilli: samples in addition obtained from fissures and approximal surfaces.

[b] Percentage of total anaerobically cultivable flora on mitis-salivarius agar.

[c] Percentage of total anaerobically cultivable flora on blood agar.

[d] Organisms not detected; 500 colonies counted on mitis-salivarius agar.

bacterial cells for oral surfaces, (ii) the number of cells available for attachment, (iii) the rate of bacterial growth, (iv) the frequency of cell transfer, (v) the time available for the transferred cells to become attached, (vi) bacterial survival during transfer, (vii) host factors that influence bacterial attachment or growth, and (viii) the composition of the host's diet. These factors govern intraoral spread as well as transfer between subjects.

Previous work on the adherence of naturally occurring and labeled *S. sanguis* and *S. salivarius* and on that of labeled lactobacilli has indicated that the affinity of *S. salivarius* and lactobacilli for teeth is comparable but is far exceeded by that of *S. sanguis* (24–26). The present data obtained with naturally occurring *S. salivarius* and lactobacilli are consistent with these findings. Also, the affinity of naturally occurring *S. mutans* was found to be higher than that of *S. salivarius* under the dietary conditions, in particular sucrose intake, of the study. This would place this species in between *S. sanguis*, and *S. salivarius* and the lactobacilli with respect to its affinity for teeth.

In earlier studies involving *Streptococcus* sp., *Veillonella*, *Neisseria*, and lactobacilli, the relative affinity of these species has been found to correlate well with the proportions in which they naturally occur in different oral sites (9). Consequently, selective bacterial attachment has been proposed as an important ecological determinant (9). The data on bacterial attachment were obtained in experiments in which the proportions of different species on the tooth surface, indicative of relative bacterial affinity, were determined on tooth surfaces exposed to in all instances high cell numbers. However, the number of cells of a given species that will adhere to oral surfaces depends not only on the affinity of the cells but also on their number available for attachment. It is known that the salivary concentrations of *S. sanguis* and *S. salivarius* are generally in the order of 10^7 to 10^8 CFU/ml (2, 7, 16), whereas, as found in previous studies and the present work, those of *S. mutans* and the lactobacilli are much lower, exhibiting a wide range from undetectable to about 10^6 CFU/ml (7, 23, 26). In this study, the salivary concentrations of *S. mutans* were 10^4 CFU/ml or less and those of the lactobacilli were 10^5 CFU/ml or less in over half of the subjects studied.

It may be assumed a priori that these differences in salivary concentrations should influence the attachment of these species. Of special interest, however, is that for each of the studied organisms a certain critical salivary concentration was found below which they could not be detected on teeth after oral exposure. This concentration is surprisingly high and is in the order of 10^4 to 10^5 CFU/ml for *S. mutans*, *S. salivarius*, and lactobacilli after a 2- to 3-h exposure. It is unlikely that this is due to bacterial death upon contact with the tooth surface since these organisms survive well in saliva (26; J. van Houte, unpublished data) that serves as a source of certain components that become sorbed on cleaned tooth surfaces, thereby forming a layer on which the bacteria subsequently settle (9).

It is recognized that the methods employed do not provide absolute certainty about the absence of bacteria on the teeth and that therefore the critical salivary concentrations may have been lower than observed. Nevertheless, the data suggest that the numbers of *S. mutans* and lactobacilli generally found in human saliva, in contrast to those of *S. sanguis*, are so low that, given their affinity, successful attachment to nonretentive tooth surfaces is of only a very low probability. Thus, to account for the inability of *S. mutans* and lactobacilli to colonize the tooth surface ubiquitously as ob-

served in previous (11, 15, 20–22; Shklair et al., abst. no. 72, Int. Ass. Dent. Res., 1972) and the present work (Table 6), and consequently to reach salivary concentrations in the same order as those of *S. sanguis*, it is unnecessary to postulate that this is due to their inability to grow on the tooth surface. The validity of this concept is strengthened by recent studies on the nutrition of oral streptococci that have revealed their simple, similar requirements (3, 19). Also, *S. mutans* and the lactobacilli are known to accumulate in large numbers in the mouth when conditions for their retention are made more favorable, such as an increased sucrose consumption in the case of *S. mutans* (6, 9) or the insertion of mechanical devices in case of the lactobacilli (26).

The recovery of bacteria from the teeth in the present study and in earlier ones (24–26), does not appear to necessarily imply that the organisms were firmly attached. The adherence of oral bacteria such as the streptococci to oral surfaces seems to involve an initial, reversible phase of sorption prior to irreversible sorption (9). A constant bacterial exchange occurs between the surfaces and their environment. Many bacteria will become temporarily reversibly sorbed, but only a small proportion of the total number of cells that come in contact with the surfaces will become firmly attached. Consequently, probably only salivary concentrations of *S. mutans* or lactobacilli, even considerably exceeding those observed in the present study to be required for their isolation from nonretentive tooth surfaces, will lead to firm attachment within a reasonably short period of time.

Based essentially on the interrelated parameters bacterial affinity and bacterial numbers available for attachment, the following hypothesis may now be proposed to explain the differences between various *Streptococcus* sp. and the lactobacilli in their ability to colonize the mouth. The oral introduction of *S. mutans* and lactobacilli, due to their low affinity, will only sporadically result in their firm attachment to and subsequent colonization of the teeth. This, instead of a low growth rate on the teeth, will lead to low salivary concentrations that in turn will unfavorably influence, or make all together impossible, the spread of these organisms to other tooth surface sites. In contrast, *S. sanguis* and *S. salivarius* have a high relative affinity for the teeth and the dorsum of the tongue, respectively (25, 26). This characteristic, together with the resulting high salivary concentrations, will greatly favor not only their initial oral establishment but also the rate of their spread to other tooth surface sites or other parts of the tongue. This concept may explain previous observations on the lack of intraoral spread (D. C. Edman et al., abst. no. 73, Int. Ass. Dent. Res., 1973) or transmission in the human population (14) of artificially introduced labeled *S. mutans*. In both studies, the limited extent of its establishment on the teeth probably did not lead to sufficiently high salivary concentrations so as to permit successful transfer. So far, major reservoirs of *Streptococcus* sp. have not been identified outside man and some animals (1). This suggests that not only the intraoral spread of these organisms but also their intersubject transmission is for a major part accomplished via saliva, and that its extent is similarly influenced by their salivary concentration. This reasoning should to a lesser extent apply to the lactobacilli that are more widespread in nature (18).

Differences between the attachment of *S. mutans*, *S. sanguis*, and lactobacilli to teeth may be due to differences in the initial reversible phage of sorption. Also, the oral establishment of *S. mutans* on teeth in man and animals is specifically dependent on dietary sucrose. The unique role of sucrose seems to be related to the fact that it specifically permits the synthesis of extracellular glucans that mediate the initial attachment of *S. mutans* to the teeth as well as its accumulation (9). On the other hand, host polymers, i.e., salivary glycoproteins, seem to play an important role in the attachment of *S. sanguis* (9). Because dietary sucrose intake occurs only intermittently but the salivary constituents are present continuously, the differences in attachment between both organisms may in addition be due to differences in the frequency of occurrence of conditions conducive to firm attachment. The lactobacilli seem to be, even more so than *S. mutans*, dependent on retentive areas for their oral maintenance. Although a general, significant influence of dietary carbohydrate on their presence in the mouth has been demonstrated (13), specific dietary carbohydrates or host substances such as salivary components have so far not been clearly recognized to be of significance in their attachment to teeth. It may be speculated that generally neither the host nor the diet provides these organisms with substances that permit their firm attachment.

In the present study only relatively nonretentive tooth surfaces have been studied. Because *S. mutans* preferentially colonizes areas c teeth considered retentive such as fissures and approximal surfaces, it may be deduced that

this organism establishes in these sites with a higher efficiency than in others. It would be of interest to compare the efficiency with which *S. mutans* colonizes retentive and nonretentive tooth surface areas in relation to its salivary concentration. Such a study may provide information concerning the feasibility of interfering with the intraoral spread of *S. mutans* or its transmission from infected to uninfected subjects by decreasing the salivary levels of the organism via the filling of carious lesions and its eradication from infected sites by means of antibacterial agents or as the result of the use of occlusal or other types of sealants.

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