Colonization of Teeth in Humans by *Streptococcus mutans* as Related to Its Concentration in Saliva and Host Age

S. DUCHIN† AND J. VAN HOUTE*

Forsyth Dental Center, Boston, Massachusetts 02115

Received for publication 11 October 1977

The relationship of the salivary concentration of *Streptococcus mutans* and host age to the colonization of this organism on erupting teeth was studied in humans. Plaques were obtained from fissures and buccal surfaces of erupting permanent first and second molars of children 6 to 7 and 11 to 14 years old, respectively. In subjects of both age groups with salivary *S. mutans* concentrations below 5×10^2 colony-forming units per ml, the organism was detected on only a few of the tooth surfaces; at concentrations of 5×10^2 to 4.9×10^4 colony-forming units per ml more than half of the surfaces and at concentrations of 5×10^4 colony-forming units per ml or higher most of the surfaces were colonized by *S. mutans*. The frequency of detection and concentration of *S. mutans* in plaque as well as its concentration in saliva were higher in the case of the older children. However, when younger and older children with similar salivary *S. mutans* concentrations were compared, *S. mutans* was more frequently isolated from plaque from older children only in the case of children with below 5×10^2 colony-forming units per ml of saliva. Eleven of the 64 children studied had low or undetectable *S. mutans* levels in plaque and saliva. The salivary *S. mutans* levels of the parents of these children were lower than those of parents of a group of children with normal oral *S. mutans* levels.

An earlier in vivo study with humans has shown that *Streptococcus mutans* can only be detected on smooth tooth surfaces after their cleaning and subsequent exposure for a few hours to the oral environment if the concentration of this organism in saliva exceeds a certain level (25). The existence of threshold levels of *S. mutans* in saliva below which its intraoral spread is greatly decreased opens possibilities for interference with its colonization of teeth. In the present study, the relationship between the concentration of *S. mutans* in saliva and the infection of teeth by this organism was evaluated further. The presence of *S. mutans* was determined in plaques obtained from fissures and buccal surfaces of erupting permanent first and second molars of children 6 to 7 and 11 to 14 years old, respectively, with varying *S. mutans* levels in saliva.

By comparing the colonization of *S. mutans* on the selected teeth of the children of both age groups, information was also obtained about the influence of host age on oral bacterial colonization. Recent studies suggest that the susceptibility of laboratory rats to infection by *S. mutans* and *Actinomyces viscosus* varies with their age

(26; S. Brecher and J. van Houte, Int. Assoc. Dent. Res., special issue B, Abstr. no. 462, 1976). Studies of the acquisition of the oral flora by humans (for review, see reference 19) and the transmission of a *Lactobacillus* species between monkeys (2) also suggest changes in host susceptibility during aging.

MATERIALS AND METHODS

Subjects and teeth for sampling. Children with erupting upper and lower permanent first and second molars were selected during a screening examination at the public schools in Milton, Mass., and at Children's Hospital in Boston, Mass. About half of the teeth were in the early phase of eruption, i.e., no part of the tooth was more than 2 mm above the gingiva. The remaining teeth did not extrude more than 4 mm from gingiva, i.e., up to about half of their crown length. Based on recent studies (Moorrees, Forsyth Dental Center, Boston, Mass., personal communication), the eruption process may be estimated to have been in progress in the case of the upper and lower first molars for, respectively, 1.4 and 1.7 months (up to 2 mm) and 4.1 and 6.6 months (2 to 4 mm erupted); for the upper and lower second molars the figures are, respectively, 2.1 and 3.6 months (up to 2 mm) and 5.2 and 7.8 months (2 to 4 mm erupted). None of the selected teeth made direct contact with teeth in the opposing jaw.

From each child a few milliliters of unstimulated whole saliva was obtained. Plaque samples were col-

† Present address: University of Pittsburgh, School of Dental Medicine, Department of Periodontology, Pittsburgh, PA 15261.

lected with dental explorers. With a few exceptions, the fissures and the gingival area of the accessible buccal surface of each tooth were sampled. Fifty-eight occlusal and 58 buccal surfaces of first molars were sampled from 30 subjects. In the case of the second molars, 48 occlusal surfaces were sampled from 34 subjects and 46 buccal surfaces were sampled from 31 subjects. All 210 samples were scored as positive or negative for *S. mutans*. The presence of *S. mutans* in 141 of the 210 samples was also calculated as a percentage of the total cultivable flora; these samples included 38 samples from fissures and 37 samples from buccal surfaces of first molars from 19 subjects, 33 samples from fissures of second molars from 24 subjects, and 33 samples from buccal surfaces of second molars from 23 subjects.

Eleven of the 64 children that were studied had very low or undetectable *S. mutans* levels. Seven of these children were 6 to 7 and four were 11 to 14 years old. None of these children consumed a special diet. None had received long-term antibiotic therapy, except one 7-year-old child who had been treated with ampicillin for 1.5 months 2 years before the study and another who was treated extensively with various antibiotics during the first 3 years of life and still occasionally used tetracycline or penicillin. None of the 11 children consumed fluoridated drinking water. Four of the children had recently been treated with topical fluoride, 2 had been treated in the past, and 5 had not been treated. Nine of the 11 children had received fluoride supplements in tablet or liquid form; 3 had received supplements since birth and 6 had received them for varying periods during life.

Sample dispersion and plating. All plaque samples were placed in small screw-capped vials with 2.0 ml of VMGII transport medium (16). Dispersion of microorganisms in plaque and saliva was carried out with a Vortex Genie mixer (Scientific Products, American Hospital Supply Co., Evanston, Ill.) for 30 s at setting 6, using glass beads for plaque samples (11). Serial dilutions were made in quarter-strength Ringer solution (24) with homogenization on the Vortex mixer for 10 s between successive dilutions. Samples (0.1 ml) of undiluted and appropriately diluted suspensions of

the 141 plaque samples were spread in duplicate on MSB agar selective for *S. mutans* (10) and on blood agar, consisting of Trypticase soy agar (Baltimore Biological Laboratory, Cockeysville, Md.) with 5% sheep blood (Scott Laboratories, Inc., Fiskeville, R.I.), for total viable counts. Samples (0.1 ml) of undiluted suspensions of the remaining plaque samples and of undiluted and appropriately diluted suspensions of saliva were cultured only on MSB agar. Dispersion and plating of each sample were completed within 6 h of collection. All plates were incubated anaerobically in Brewer jars containing an atmosphere of 80% N₂-10% H₂-10% CO₂ at 37°C for 2 days. *S. mutans* was identified by characteristic colonial morphology (10) supplemented, if necessary, with suitable biochemical tests (3).

Plaque samples were scored as positive when one colony or more of *S. mutans* were present on the duplicate agar plates on which undiluted samples were cultured. Thus, the theoretical detection limit of the organism in each plaque sample was 10 colony-forming units (CFU), since two samples of 0.1 ml from a total volume of 2 ml were cultured. However, it is possible that in this evaluation plaque samples were scored as positive due to contamination with *S. mutans*-infected saliva. In a second evaluation, therefore, plaque samples were only scored as positive when a total of more than 40 colonies were present on the duplicate plates on which undiluted samples were cultured. Such counts could only result from contamination of the small plaque samples with 4 µl of saliva containing 10⁵ CFU/ml, which is highly unlikely.

RESULTS

The proportions of *S. mutans* in the plaque samples from occlusal and buccal surfaces of the first molars were lower than those in the samples from the second molars (Table 1) (rank-sum test [17]; occlusal surfaces, $P < 0.005$; buccal surfaces, $P < 0.05$). The first molars were exposed to lower salivary *S. mutans* concentrations than were the second molars (median test [17]; occlusal and buccal surfaces, $P < 0.005$). There was

TABLE 1. Concentrations of *S. mutans* in 141 plaque samples obtained from occlusal and buccal surfaces of first and second molars and in saliva from the same subjects

Sample group		<i>S. mutans</i> concn in:			
		Plaque (%) ^a		Saliva (CFU/ml)	
Molar	Surface	Mean	Median	Mean	Median
First	Occlusal	8.0 (0-83.3) ^b	0.02	1.1 × 10 ⁵ (0-8.5 × 10 ⁵)	1.5 × 10 ³
	Buccal	1.2 (0-14.0)	0.005	1.2 × 10 ⁵ (0-8.5 × 10 ⁵)	1.8 × 10 ³
Second	Occlusal	13.7 (0-100.0)	3.0	1.5 × 10 ⁵ (0-9.6 × 10 ⁵)	2.3 × 10 ⁴
	Buccal	3.3 (0-24.0)	0.1	1.5 × 10 ⁵ (0-9.6 × 10 ⁵)	2.3 × 10 ⁴

^a Percentage of total viable count on blood agar.

^b Range of arithmetic mean.

no correlation between the concentrations of *S. mutans* in saliva and in plaque from first or second molars (Table 1) (coefficient of correlation [17]; occlusal or buccal surfaces, $P > 0.05$).

S. mutans was more frequently detected in plaques from second molars than in those from first molars (Table 2) (chi-square test; occlusal surfaces, $P < 0.005$; buccal surfaces, $P < 0.01$). Since the second molars were exposed to saliva with higher *S. mutans* concentrations (Table 1), the frequency of isolation of *S. mutans* from first and second molars was also evaluated in relation to salivary concentration (Table 3). In subjects with less than 5×10^2 CFU/ml of saliva, more first-molar than second-molar samples were negative for *S. mutans* (chi-square test: $P < 0.01$, both types of scores, A and B). In this category, *S. mutans* was not detected in saliva or plaque from 8 of the 10 subjects from whom first molars were sampled and from 3 of the 6 subjects from whom second molars were sampled; in subjects with *S. mutans* in saliva, the isolation frequency of *S. mutans* in plaque was: first molars, 2+ and 10-; second molars, 5+ and 3-. In subjects with higher *S. mutans* concentrations in saliva, there was no difference in isolation frequency of *S. mutans* between samples from first and second molars (chi-square test: $P > 0.05$, both types of scores, A and B). Table 3 also indicates that *S. mutans* was detected more frequently in plaque from teeth that had been longer in the process of eruption.

The frequency of detection of *S. mutans* in plaques from first and second molars of subjects with less than 5×10^2 CFU/ml of saliva was very low; it was much higher in subjects with salivary *S. mutans* concentrations of 5×10^2 to 4.9×10^4 CFU/ml, and it was very high at *S. mutans* concentrations of 5×10^4 to 5×10^6 CFU/ml of saliva (Table 3, both types of score, A and B). The isolation frequency of *S. mutans* in plaques from occlusal or buccal tooth surfaces increased gradually with increasing salivary concentration (Table 4). The correlation ratio (17)

TABLE 2. Detection^a of *S. mutans* in 141 plaque samples obtained from occlusal and buccal surfaces of first and second molars

Sample source		No. of samples:	
Molar	Surface	+	-
First	Occlusal	15	23
	Buccal	15	22
Second	Occlusal	29	4
	Buccal	25	8

^a Scores do not differ whether a positive score (+) is defined as ≥ 1 CFU or > 40 CFU on duplicate MSB plates on which undiluted samples were cultured.

used for all 106 occlusal and 104 buccal samples separately indicated that this trend was significant for both types of surfaces ($P < 0.005$, both types of scores). Most samples recovered from occlusal surfaces were positive for *S. mutans* at salivary concentrations of 5×10^2 to 4.9×10^3 CFU/ml, whereas most samples from buccal surfaces were positive only at 10-fold-higher salivary concentrations. However, only at salivary *S. mutans* levels of 5×10^3 to 4.9×10^4 CFU/ml or higher was it more likely that occlusal or buccal plaque samples would be positive for *S. mutans* (chi-square one-sample test (18): $P < 0.05$, both score definitions).

S. mutans was not detected in the saliva or plaque of 11 of the 64 children. A sample of saliva and 2 to 8 plaque samples from the same tooth surfaces sampled before were obtained 3 to 9 months later. *S. mutans* was not detected in saliva and plaque of five of the 6- to 7-year-old children and one older child, and the highest salivary *S. mutans* concentration was 7.0×10^3 CFU/ml (Table 5, group I). The median and mean plaque concentrations of these children were 0 and 0.1%, respectively (data not shown). The mean concentration of *S. mutans* in saliva of the parents of the 11 children was in the order of 10^4 CFU/ml (Table 5), and the organism was undetectable in saliva of 3 of the 22 parents. Both parents from children with detectable *S. mutans* in saliva also harbored the organism; both parents from 1 child with undetectable *S. mutans* in saliva were also negative for the organism. In contrast, the mean salivary concentration of mothers from a group of 9 children examined previously (23) was in the order of 10^5 CFU/ml (Table 5, group II) and was significantly higher than that of either the mothers or the fathers of the 11 children in group I (Fischer exact probability test [18]: $P < 0.05$). Seven of the 9 children in group II were infected with *S. mutans* and had salivary *S. mutans* concentrations in the order of 10^3 to 10^4 CFU/ml.

Clinical examination of the 11 children with low *S. mutans* levels with explorer and mirror revealed the absence of fillings or caries lesions in the 6 children without detectable *S. mutans*; in contrast, all 5 children with detectable *S. mutans* had fillings and/or caries lesions.

DISCUSSION

A significant positive correlation was observed between the concentrations of *S. mutans* in saliva and its isolation from the selected tooth surfaces; there was no positive correlation between the concentrations of *S. mutans* in saliva and in plaque. At salivary concentrations lower than 5×10^2 CFU/ml, most surfaces of the

TABLE 3. *Detection of S. mutans in all plaque samples obtained from first and second molars of subjects categorized according to S. mutans concentration in saliva*

Score type ^a	Range of salivary concn (CFU/ml)	First molar				Second molar			
		Estimated time of eruption (mo)		No.		Estimated time of eruption (mo)		No.	
		Upper	Lower	+	—	Upper	Lower	+	—
A	0-4.9 × 10 ²	1.4	1.7	0	24	2.1	3.6	5	7
		4.1	6.6	2	11	5.2	7.8	0	0
	5 × 10 ² -4.9 × 10 ⁴	1.4	1.7	15	7	2.1	3.6	22	6
		4.1	6.6	21	9	5.2	7.8	16	2
	5 × 10 ⁴ -5 × 10 ⁶	1.4	1.7	7	0	2.1	3.6	16	1
		4.1	6.6	16	2	5.2	7.8	21	0
B	0-4.9 × 10 ²	1.4	1.7	0	24	2.1	3.6	5	7
		4.1	6.6	2	11	5.2	7.8	0	0
	5 × 10 ² -4.9 × 10 ⁴	1.4	1.7	11	11	2.1	3.6	18	10
		4.1	6.6	19	11	5.2	7.8	16	2
	5 × 10 ⁴ -5 × 10 ⁶	1.4	1.7	6	1	2.1	3.6	15	2
		4.1	6.6	15	3	5.2	7.8	20	1

^a Positive score defined as ≥1 CFU (A) or >40 CFU (B) on duplicate MSB plates on which undiluted samples were cultured.

TABLE 4. *Detection of S. mutans in all plaque samples obtained from occlusal and buccal surfaces in relation to its concentration in saliva*

Range of salivary concn (CFU/ml)	No. + or - by score type: ^a							
	A				B			
	Occlusal		Buccal		Occlusal		Buccal	
	+	-	+	-	+	-	+	-
0	1	14	0	15	1	14	0	15
1-4.9 × 10 ¹	0	1	0	2	0	1	0	2
5 × 10 ¹ -4.9 × 10 ²	3	5	3	5	3	5	3	5
5 × 10 ² -4.9 × 10 ³	12	5	6	11	10	7	5	12
5 × 10 ³ -4.9 × 10 ⁴	28	5	26	5	25	8	22	9
5 × 10 ⁴ -4.9 × 10 ⁵	17	2	18	0	15	4	16	2
5 × 10 ⁵ -5 × 10 ⁶	12	1	13	0	12	1	13	0

^a Positive score defined as ≥1 CFU (A) or >40 CFU (B) on duplicate MSB plates on which undiluted samples were cultured.

TABLE 5. *Concentration of S. mutans in saliva of parents of children with low or average S. mutans levels*

Group	n	S. mutans concn (CFU/ml)		
		Mean ^a	Range	Median
I. Children with low <i>S. mutans</i> levels	11	1.1 × 10 ³	0-7.0 × 10 ³	0
Mothers	11	6.1 × 10 ⁴	0-2.7 × 10 ⁵	8.0 × 10 ³
Fathers	11	7.2 × 10 ⁴	0-3.3 × 10 ⁵	4.8 × 10 ⁴
II. Other children ^b	9	1.2 × 10 ⁴	0-3.5 × 10 ⁴	5.0 × 10 ³
Mothers	9	5.5 × 10 ⁵	6.9 × 10 ⁴ -2.0 × 10 ⁶	2.0 × 10 ⁵

^a Arithmetic mean.

^b Data from van Houte and Duchin (23).

erupting teeth were negative for *S. mutans*, although they had been exposed to the oral environment for periods of over 0.5 year; at higher salivary concentrations, the majority of the surfaces were infected. There was a not statistically significant tendency for occlusal surfaces to be-

come infected at lower salivary *S. mutans* concentrations than those associated with infection of buccal surfaces.

These findings are consistent with the results from a recent study of the relation between the concentration of *S. mutans* in saliva and its

colonization in sterile Mylar fissures (M. Svanberg and W. J. Loesche, Int. Assoc. Dent. Res., special issue A, Abstr. no. 455, 1977) and with the results of an earlier study in which *S. mutans* was not recovered from cleaned buccal tooth surfaces of humans within 3 h after cleaning unless its concentration in saliva was about 10^4 CFU/ml or higher (25). Since the *S. mutans* concentrations in saliva are determined by the cell populations on the teeth and other oral surfaces, it is possible that the relationship between salivary concentration and tooth surface infection observed in the present study has been influenced by variation among subjects with different salivary concentrations in their susceptibility to infection.

Recent studies have shown that the application of disinfectants, such as iodine or stannous fluoride, to teeth is effective in reducing *S. mutans* populations from many of the treated surfaces for prolonged periods of time (8, 12). The present findings suggest that these procedures may be even more effective if disinfection also leads to a decrease of the *S. mutans* concentrations in saliva below a level at which the probability of reinfection appears to be greatly diminished.

In view of the early onset of dental caries, attempts to interfere with oral infection by suspected dental pathogens, such as *S. mutans*, should preferably be made early in life. Information about the susceptibility of human teeth to *S. mutans* at various ages is of interest with respect to the timing, duration, and prognosis of antibacterial therapy. Infants may acquire *S. mutans* shortly after tooth eruption (1). Fissures of natural teeth or Mylar fissures initially free of bacteria, when placed in the mouth of human adults, become colonized by *S. mutans* within days (20–22; M. Svanberg and W. J. Loesche, Int. Assoc. Dent. Res., special issue A, Abstr. no. 455, 1977). However, no definite conclusions about the relative susceptibility of the teeth of infants or adults can be drawn, due to the lack of information about the extent of exposure to the organism.

The present study does not indicate major differences between 6- to 7- and 11- to 14-year-old children in their susceptibility to infection by *S. mutans*. *S. mutans* was more frequently detected on the tooth surfaces of the older children (Table 2). However, these children had higher *S. mutans* concentrations in saliva, which, in absence of direct contact of the selected teeth with other teeth, probably constituted a major source of infection. Comparison of children with salivary *S. mutans* concentrations of the same order showed that this difference was only discernable in the case of children with

the lowest salivary *S. mutans* levels. Since the second permanent molars erupted slower than the first permanent molars, this small difference in the isolation frequency of *S. mutans* between these teeth may have been at least partly due to differences in exposure to the organism. This is supported by the fact that the frequency of detection of *S. mutans* on first or second molars was often increased in the case of molars that were longer in the process of eruption (Table 3).

The effect of host age on infection by *S. mutans* was not studied in a similar manner in the case of adults, due to the unfavorable eruption pattern of the third molar. Although direct information about the relative susceptibility of infants, children, and adults to infection by *S. mutans* is lacking, the evidence suggests that this organism will readily colonize newly exposed teeth of children and adults in the presence of suitable salivary levels. Thus, efforts to prevent infection or reinfection of teeth by *S. mutans* cannot be reduced when humans become older.

The salivary *S. mutans* concentrations of the parents of 11 children with low or undetectable *S. mutans* in plaque or saliva were low compared with those of parents of children with normal salivary *S. mutans* levels. It is unlikely that the low *S. mutans* levels of these children were due to the use of antibiotics (6; W. J. Loesche, D. R. Bradbury, and M. P. Woolfolk, Am. Assoc. Dent. Res., special issue, Abstr. no. 335, 1975) or to the administration of fluoride in drinking water (7), as supplements (J. van Houte, R. Aasenden, and T. C. Peebles, Arch. Oral Biol., in press), or via local application to the teeth (14, 15, 27). A positive correlation between the numbers of *S. mutans* in saliva of mothers and their children has recently been observed by others (B. Köhler and D. Bratthall, Int. Assoc. Dent. Res., special issue A, Abstr. no. 457, 1977). These findings should be further substantiated by studying other parent-child combinations from different locations. In 6 of the 11 children, *S. mutans* could not be isolated from plaque or saliva. These children had no fillings or detectable caries lesions. In contrast, there was evidence of caries activity in the other children in which *S. mutans* was present in low levels. This finding is consistent with data from other studies (9), which indicate a positive correlation between *S. mutans* and caries activity.

S. mutans was not detected in about 10% of the children that were studied. The available data indicate that the isolation frequency of *S. mutans* with MSB agar is about 50% in the case of dentulous infants up to about 14 months of age (1), increases to about 80% with children 2.5 to 7 years of age (23; this study) and to over 90% with children 9 to 14 years of age (13; this study),

and is close to 100% in older children and adults (25). The low frequency of isolation of *S. mutans* from children up to 5 years old reported by others (4, 5) is probably due to the use of different sampling methods or less sensitive isolation media.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant DE-02847 from the National Institute of Dental Research.

LITERATURE CITED

- Berkowitz, R. J., H. V. Jordan, and G. White. 1975. The early establishment of *Streptococcus mutans* in the mouths of infants. Arch. Oral Biol. 20:171-174.
- Bowen, W. H. 1968. Dental caries in monkeys. Adv. Oral Biol. 3:185-216.
- Carlsson, J. 1967. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites of the oral cavity in man. Odontol. Revy 18:55-74.
- Carlsson, J., H. Grahnen, and G. Jonsson. 1975. Lactobacilli and streptococci in the mouths of children. Caries Res. 9:332-339.
- Catalanatto, F. A., I. L. Shklair, and H. J. Keene. 1975. Prevalence and localization of *Streptococcus mutans* in infants and children. J. Am. Dent. Assoc. 91:606-609.
- dePaola, P. F., H. V. Jordan, and J. Berg. 1974. Temporary suppression of *Streptococcus mutans* in humans through topical application of vancomycin. J. Dent. Res. 53:108-114.
- deStoppelaar, J. D., J. van Houte, and O. Backer Dirks. 1969. The relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. Caries Res. 3:190-199.
- Gibbons, R. J., P. F. DePaola, D. M. Spinell, and Z. Skobe. 1974. Interdental localization of *Streptococcus mutans* as related to dental caries experience. Infect. Immun. 9:481-488.
- Gibbons, R. J., and J. van Houte. 1975. Dental caries. Annu. Rev. Med. 26:121-136.
- Gold, O. G., H. V. Jordan, and J. van Houte. 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. 18:1357-1364.
- Jordan, H. V., B. Krasse, and A. J. R. Möller. 1968. A method of sampling human dental plaque for certain "caries-inducing" streptococci. Arch. Oral Biol. 13:919-927.
- Keene, H. J., I. L. Shklair, and G. J. Mickel. 1977. Effect of multiple dental floss-SnF₂ treatment on *Streptococcus mutans* in interproximal plaque. J. Dent. Res. 56:21-27.
- Klock, B., and B. Krasse. 1977. Microbial and salivary conditions in 9 to 12-year-old children. Scand. J. Dent. Res. 85:56-63.
- Loesche, W. J., R. J. Murray, and J. R. Mellberg. 1973. The effect of topical acidulated fluoride on percentage of *Streptococcus mutans* and *Streptococcus sanguis* in interproximal plaque samples. Caries Res. 7:283-296.
- Loesche, W. J., S. A. Syed, R. J. Murray, and J. R. Mellberg. 1975. Effect of topical acidulated phosphate fluoride on percentage of *Streptococcus mutans* and *Streptococcus sanguis* in plaque. II. Pooled occlusal and pooled approximal samples. Caries Res. 9:139-155.
- Möller, A. J. R. 1966. Microbiological examination of root canals and periapical tissues of human teeth. Odontol. Tidskr. 74: no. 5-6, special article.
- Senders, V. L. 1958. Measurement and statistics. Oxford University Press, New York.
- Siegel, S. 1956. Nonparametrical statistics for the behavioral sciences. McGraw-Hill Book Co., New York.
- Socransky, S. S., and A. D. Manganiello. 1971. The oral microbiota of man from birth to senility. J. Periodontol. 42:485-496.
- Theilade, E., O. Fejerskov, W. Prachyabrued, and M. Kilian. 1974. Microbiologic study on developing plaque in human fissures. Scand. J. Dent. Res. 82:420-427.
- Theilade, E., R. H. Larson, and T. Karring. 1973. Microbiological studies of plaque in artificial fissures implanted in human teeth. Caries Res. 7:130-138.
- Thott, E. K., L. E. A. Folke, and O. B. Sveen. 1974. A microbiologic study of human fissure plaque. Scand. J. Dent. Res. 82:428-436.
- van Houte, J., and S. Duchin. 1975. *Streptococcus mutans* in the mouths of children with congenital sucrase deficiency. Arch. Oral Biol. 20:771-773.
- van Houte, J., R. J. Gibbons, and S. B. Banghart. 1970. Adherence as a determinant of the presence of *Streptococcus salivarius* and *Streptococcus sanguis* on the human tooth surface. Arch. Oral Biol. 15:1025-1034.
- van Houte, J., and D. B. Green. 1974. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. Infect. Immun. 9:624-630.
- van Houte, J., V. N. Upeslakis, and S. Edelstein. 1977. Decreased oral colonization of *Streptococcus mutans* during aging of Sprague-Dawley rats. Infect. Immun. 16:203-212.
- Woods, R. 1971. The short-term effect of topical fluoride applications on the concentration of *Streptococcus mutans* in dental plaque. Aust. Dent. J. 16:152-155.