INFECTION AND IMMUNITY, Oct. 1976, p. 1022-1027

Vol. 14, No. 4
Printed in U.S.A.

Initial Colonization of Teeth in Monkeys as Related to Diet

M. KILIAN* AND G. RÖLLA

Royal Dental College, Departments of Microbiology and Preventive Dentistry, Vennelyst Boulevard, DK-8000 Aarhus C, Denmark*

Received for publication 6 April 1976

The initial phases of plaque development on nonretentive tooth surfaces were studied bacteriologically in *Macaca irus* monkeys fed by stomach tube and provided with various oral supplements. Except for the oral implantation of *Streptococcus mutans* in some of the animals, the oral flora was not changed prior to the studies. Dental plaque was allowed to develop on initially cleaned tooth surfaces for 3 to 5 h. Plaque samples were collected and cultured on a number of selective and nonselective agar media, and several hundred isolates from each sample were isolated and identified. The numerically predominant organisms in initial plaque were *S. mutans*, *Streptococcus sanguis*, and *Actinomyces viscosus*. Additional organisms regularly found, but usually in smaller numbers, were *Streptococcus mitior* and a group of fastidious gram-negative rods including *Haemophilus* species, *Eikenella corrodens*, and *Actinobacillus actinomycetem-comitans*. The colonization of *S. mutans* was dependent on sucrose and occurred at the expense of *S. sanguis*. In these experiments *S. mutans* accounted for 25 to 65% of the primary plaque formers. All other species encountered colonized the teeth irrespective of the diet. It is postulated that the early sucrose-dependent establishment of *S. mutans* directly on the enamel pellicle plays a key role in the development of a cariogenic plaque.

The initial steps in bacterial accumulation on teeth require cell attachment to the acquired enamel pellicle sufficiently strong to withstand local cleansing forces. This colonization is known to start shortly after exposure of cleaned tooth surfaces to the oral environment (38, 40, 42). The microbial composition of the early developing plaque is believed to reflect the relative affinity of each bacterial species available for colonization as well as their numbers present in the mouth (16). Most studies of the innate affinities of oral bacterial species for particular sites within the oral cavity have been performed in vitro (18, 36) or have focused on few species (30, 31, 42, 43) without taking dietary factors into consideration. It is known that dietary carbohydrates influence the microbial composition of dental plaque in humans (4, 10, 27, 28) and in various animals (2, 26). Sucrose seems to be a major determinant of the cariogenic potential of dental plaque (15).

The purpose of the present investigation was to study the relative proportions of the components of the indigenous oral flora in monkeys colonizing tooth surfaces under various simplified dietary intakes. The initial phases of plaque development on cleaned tooth surfaces were studied bacteriologically in animals fed by stomach tube and provided with various oral supplements. Except for the oral implantation of *Streptococcus mutans* in some of the animals, the oral flora was not changed prior to the studies.

MATERIALS AND METHODS

Animals. Three female *Macaca irus* monkeys between 4 and 8 years of age were used in this investigation. The animals were caged separately. Throughout the experimental periods, the animals were fed a complete sucrose-free diet (Complan, Glaxo, Copenhagen) by stomach tube as described by Bowen and Cornick (3). In addition to this basic diet, they received one of three different supplements by mouth for 9 to 14 days prior to each bacteriological sampling: (i) sucrose-sweetened (approximately 10% wt/vol) tap water ad libitum; (ii) casein administered twice daily plus drinking water ad libitum; (iii) drinking water ad libitum. An interval of sucrose supplementation was always alternated between periods of casein or water supplementation. Feeding of the animals by tube as well as bacteriological samplings were carried out after the animals had been tranquilized with 3 mg of Sernylan (Parke, Davis & Co., Detroit, Mich.) per kg administered intramuscularly. Two of the animals (no. 317 and no. 318) were inoculated intraorally with *S. mutans* strain GS 5 during a period of sucrose supplementation. The presence of the implanted strain was monitored culturally throughout the experiment.

Sample collection and culturing procedure. On the day of sampling, the labial surfaces of the upper and lower anterior teeth were pumiced thoroughly and flushed with sterile saline using a syringe. Bac-

teriological samples (see below) were taken to determine the efficacy of the prophylaxis. Dental plaque was then allowed to develop for 3 to 5 h by exposing the teeth to the oral environment. At the end of the experimental period, the anterior teeth were flushed vigorously with 5 ml of sterile saline to remove adherent saliva and loosely attached bacteria. After a short drying period, the labial surface of one of the central upper incisors was sampled by scraping the surface (about 0.5 cm^2) with the end of a sterile orthodontic band. Care was taken to avoid including material from the gingival area or the approximal spaces. The sample was dropped into 3 ml of cooled nutrient broth (Difco Laboratories, Detroit, Mich.) containing 0.1% cysteine hydrochloride and suspended by ultrasonic treatment for 10 s using a PG100 MSE 150-W ultrasonic disintegrator with a titan microprobe at an amplitude of 6 μm. Saliva samples (approximately 1 ml) were collected at the beginning of the plaque accumulation period. Plaque and saliva samples were diluted in cold, reduced nutrient broth, and appropriate dilutions were spread on duplicate plates of agar substrates.

Plaque samples were cultured on: (i) a nonselective tryptone-yeast extract agar medium containing glucose, starch, cysteine, menadione, and hemolyzed calf blood (20), incubated for 3 days in Baird and Tatlock jars filled with either air plus 10% CO_2 or with 10% H_2, 85% N_2, and 5% CO_2; and (ii) chocolate agar (blood agar base [Difco] with 10% heated, defibrinated horse blood), incubated in Baird and Tatlock jars filled with air plus 10% CO_2.

Saliva samples were cultured on: (i) mitis-salivarius (MS) agar (Difco) incubated for 1 day in Baird and Tatlock jars with 95% N_2 and 5% CO_2, followed by 1 day at room temperature in air.

After incubation at 37°C, the plates of the dilution yielding an appropriate number of discrete colonies (30 to 300) were selected. From tooth surface samples, all colonies (up to 100) present on one selected plate of each of aerobically incubated tryptone-yeast agar, anaerobically incubated tryptone-yeast agar, and chocolate agar were isolated. Colonies from the former medium were subcultured on the same medium. Colonies from chocolate agar plates were subcultured on horse blood agar plates supplemented with a streak of *Staphylococcus* to allow detection of symbiosis. After incubation, all isolates were Gram stained and tested for catalase production. All isolates that appeared to be streptococci were transferred to MS agar. Finally, several representatives of each of the various types were selected according to cell and colonial morphology, Gram-stain characteristics, and catalase reaction, to be purified and stored by freezing at −70°C in sealed glass ampoules.

Cultures from saliva samples on MS agar were examined for the presence of *S. mutans*. Identification of the organisms was based on their characteristic colonial morphology on the medium. Several colonies were subcultured to confirm their identity.

Identification procedures. Streptococci were identified according to Colman and Williams (6) using methods as described by Carlsson (4). Production of soluble extracellular glucan and fructan by streptococcal isolates was detected by adding 1 and 3 volumes, respectively, of absolute ethanol to supernatants of 5% sucrose broth cultures (see in vitro plaque formation, below) followed by storing at 4°C for 18 h. Actinomycetes were identified according to Cowan and Steel (8) and Cross and Goodfellow (9). Fermentation reactions were determined in the OF medium (19) and in thioglycolate broth (Difco) to which the fermentable substances were added in filter-sterilized solutions to give a final concentration in the medium of 1% (wt/vol). Urease activity was determined as described by Lautrop (29). Other tests were performed using standard procedures (8). Facultative gram-negative rods were identified according to Kilian and Schiøtt (25), using methods described by Kilian (24). Other organisms were identified according to Cowan and Steel (8). The following strains were included for reference purposes: *Streptococcus mutans* strain GS 5, *Streptococcus sanguis* strain 804, *Actinomyces viscosus* strains ATCC 15987 and WVU 371, and *Actinomyces naeslundii* strain ATCC 12014. The *Actinomyces* strains were kindly provided by G. H. Bowden, London.

In vitro plaque formation. The ability of some isolates to form in vitro plaque was studied by detecting bacterial adherence on the inside of an emptied culture vessel after vigorous rinsing with water. The basal medium employed had the following composition (per 1,000 ml of water): Trypticase (Baltimore Biological Laboratory, Cockeysville, Md.), 5 g; yeast extract (Difco), 5 g; Tween 80, 5 ml; K_2HPO_4, 5 g; $MgSO_4 \cdot 7H_2O$, 2 mg; $FeSO_4 \cdot 7H_2O$, 0.2 mg; $MnCl_2 \cdot 4H_2O$, 0.1 mg. Fastidious gram-negative rods were cultivated in Levinthal broth (24). The broth media were supplemented with 1% glucose or 5% sucrose.

RESULTS

Samples of the tooth surfaces taken immediately after the cleaning procedure consistently yielded fewer than 10^3 colony-forming units. After a 3- to 5-h exposure to the oral environment, approximately 10^5 to 10^6 colony-forming units were recovered. The anaerobe-to-aerobe recovery ratio on the tryptone-yeast extract medium was about 1:1, and no qualitative differences were noted between the growth on anaerobically and aerobically incubated plates. Chocolate agar yielded a somewhat lower number of colonies (1:3 to 1:10); however, gram-negative fastidious rods were relatively more numerous.

Proportion of organisms. Calculations of the microbial composition of the plaque samples were based on the identified isolates (Table 1). The percentages presented in Table 1 represent the species distribution of these isolates. A correction factor was included to take into account dilution factors and the proportion of colony-forming units isolated from the respective media. From chocolate agar plates, only isolates that did not grow on the nonselective medium were included in these calculations. A low

number of bacterial species were predominant and consistently present in the samples. These included *S. sanguis*, *A. viscosus*, and *S. mutans*. The latter species, however, was only recovered from tooth surfaces of animals originally inoculated with *S. mutans* and only when these animals were provided with sucrose in the drinking water. In these experiments *S. mutans* accounted for 25 to 65% of the isolates. The presence of *S. mutans* was concomitant with a significant decrease in the absolute and relative number of *S. sanguis*. In contrast, the fluctuations in the proportional presence of *A. viscosus* seemed to be unaffected by changes in the dietary regime and by the presence of other specific species. In addition to the three species mentioned, *Streptococcus mitior* was isolated from all samples. With the exception of samples from experiments with water as the only oral intake, the proportion made up by this species was low (median, 4%). However, in the former experiments *S. mitior* accounted for 20 to 46% of the isolates. This increase was followed by a relative decrease in the number of *S. sanguis*. *Streptococcus salivarius* was isolated from the tooth surfaces of one of the animals (no. 315) in all experiments and in numbers corresponding to 3 to 13% of the isolates.

Gram-negative rods comprised 4 to 35% (median, 8%) of the isolates. The group included *Eikenella corrodens*, *Haemophilus* species, and *Actinobacillus actinomycetem-comitans*. With the exception of *E. corrodens*, the majority of these organisms were isolated from chocolate agar.

The remaining 5 to 15% of the isolates (median, 7%) included neisseriae, *Staphylococcus epidermidis*, *Streptococcus milleri*, *Actinomyces naeslundii*, and unidentified bacteria, the first group being the most frequent.

In saliva samples taken before the 3- to 5-h experimental period, *S. mutans* accounted for 1 to 8% of the total number of colony-forming units on MS agar (Table 1).

Characteristics of isolates. The characteristics of the *Streptococcus* species matched those described in the literature (6, 8). All isolates of *S. salivarius* from one of the animals (no. 315), however, formed very rough and adherent colonies on MS agar. After some transfers in the laboratory, the colonies often changed to the characteristic smooth-colony type. The isolates were α-hemolytic on horse blood agar and fermented glucose and sucrose but not sorbitol, mannitol, or inulin. Esculin, but not arginine, was hydrolyzed. Soluble extracellular glucan was produced in large amounts in 5% sucrose broth. Fructan was produced in smaller amounts. After conversion into the smooth colony type, the production of glucan was significantly reduced.

The isolates identified as *A. viscosus* were gram-positive facultative, branching rods that grew slightly better in the presence of 10% CO_2. They produced catalase and fermented glucose, fructose, galactose, mannose, lactose, maltose, sucrose, inositol, melibiose, and trehalose but not arabinose, rhamnose, ribose, xylose, sorbitol, salicin, lactate, sodium acetate, or pyruvic acid. Cellobiose, dextrin, adonitol, and glyco-

TABLE 1. *Microbial composition of 3- to 5-h smooth-surface plaque and salivary concentration of S. mutans in tube-fed monkeys as related to the oral intake of sucrose, casein, or water*

Isolates examined	Sucrose (%)						Casein (%)			Water (%)		
	315[a] (197)[b]	315 (153)	317[c] (225)	317 (214)	318[c] (165)	318 (186)	315 (197)	317 (194)	318 (202)	315 (168)	317 (140)	318 (113)
S. mutans	–	–	40[d]	25	65	34	–	–	–	–	–	–
S. sanguis	48	51	8	6	6	8	48	66	32	19	19	23
S. mitior	8	3	4	<1	3	4	20	3	4	35	20	46
S. salivarius	8	7	–	–	–	–	13	–	–	3	–	–
A. viscosus	19	23	20	59	14	11	2	19	43	24	44	2
E. corrodens	–	4	4	–	2	14	–	–	5	5	2	3
Haemophilus species	4	4	12	4	4	6	10	1	4	9	3	8
A. actinomycetem-comitans	2	1	4	–	1	15	–	6	4	–	1	3
Others	11	7	8	6	5	7	7	5	8	5	11	15
Salivary *S. mutans* as percentage of streptococci	–	–	8	5	4	4	–	3	1	–	3	7

[a] Animal no. 315 was not infected with *S. mutans*.
[b] Number of isolates examined.
[c] Animals no. 317 and no. 318 were infected with *S. mutans* strain GS 5.
[d] Percentages were corrected for dilution factors (see text).

gen were fermented by some isolates in OF medium but not in thioglycolate broth. Esculin was hydrolyzed, but indole and urease tests were negative. Nitrate was reduced by some of the isolates. Glucose fermentation products were lactate, acetate, and succinate.

All *Haemophilus* isolates were independent of the X-factor (hemin). A speciation was not pursued. The characteristics of strains identified as *E. corrodens* and *A. actinomycetem-comitans* were similar to those reported previously (25).

In vitro plaque formation. A significant proportion of the isolates formed plaque in vitro. Isolates of some species formed plaque only in sucrose broth, whereas others formed plaque in both sucrose and glucose broth (Table 2).

DISCUSSION

The data obtained in this study are consistent with the concept of selective bacterial attachment to oral surfaces (16). Only a very few species were found consistently on the cleaned teeth after oral exposure for 3 to 5 h. The numerically predominant organisms in the initial plaque were *S. mutans*, *S. sanguis*, and *A. viscosus*. Additional organisms regularly found, but usually in smaller numbers, were *S. mitior* and a group of fastidious gram-negative rods including *Haemophilus* species, *E. corrodens*, and *A. actinomycetem-comitans*. In accordance with previous observations of early plaque formation in humans (37, 40, 42), facultative streptococci constituted 35 to 80% of the total number of bacteria recovered.

Few but marked differences were observed in the bacterial populations colonizing the teeth under the influence of the different dietary conditions. Withdrawal of sucrose from the drinking water resulted in complete absence of detectable *S. mutans*, although the salivary concentrations of this organism remained unaffected. The unchanged salivary counts of *S. mutans* in periods of casein or water supplementation were somewhat unexpected. However, since only the anterior teeth were cleaned during the study, a reservoir of *S. mutans* probably existed in other areas of the dentition throughout such periods.

TABLE 2. *Ability of bacterial isolates from 3- to 5-h dental supragingival plaque to form plaque in vitro when cultivated with sucrose or glucose*

Species	Isolates examined (no.)	Plaque-forming isolates (no.)	
		Sucrose	Glucose
S. mutans, strain GS 5 (reisolates)	30	30	0
S. sanguis	38	18	0
S. salivarius[a]	6	6	0
A. viscosus	31	16	8
Haemophilus species	30	20	20
A. actinomycetem-comitans	24	24	24
Neisseria species	8	5	5
S. epidermidis	12	7	7

[a] Rough colony type.

A variety of in vitro and in vivo studies have shown that *S. mutans* possessed a rather feeble capacity to adsorb to teeth, relative to organisms such as *S. sanguis* or *S. mitior* (31, 36, 43). However, as shown in the present study, the ability of *S. mutans* to colonize nonretentive tooth surfaces is significantly improved by the presence of sucrose. It is known that *S. mutans* synthesizes extracellular glucans and fructans specifically from sucrose. The former polysaccharide is involved in the ability of this organism to adhere to teeth and other hard surfaces (16). The observed low salivary concentrations of this organism, relative to other streptococci, indicate that, in the presence of sucrose, the affinity of *S. mutans* for nonretentive tooth surfaces is superior to that of other streptococcal species. This is further substantiated by the fact that the sucrose-dependent colonization of *S. mutans* occurred at the expense of *S. sanguis*. These findings adequately explain the observed sucrose promotion of *S. mutans* implantation in the mouths of humans (27) and various animals (26). The inverse relationship between proportions of *S. mutans* and *S. sanguis* has been observed previously in mature dental plaque in primates (7, 10, 39).

The presence of teeth is known to be a prerequisite for the oral establishment of *S. sanguis* and *S. mutans* early in life (16). However, whereas *S. sanguis* generally becomes established within the first months after the eruption of teeth, the appearance of *S. mutans* is delayed and it is often absent (1, 5). Furthermore, there is evidence that *S. mutans* is not readily transmitted from one individual to another (22) or even from one tooth surface to another within the same mouth (11). There may be several reasons for this apparently feeble capacity of *S. mutans* to become established in dental plaque under natural conditions, in spite of the high affinity observed in this study. It is possible that an extremely high consumption of sucrose, as in the present study, or a large inoculum (43) is required for implantation. In addition, some recent findings from experimental plaque formation in germ-free and specific pathogen-free rats indicate that the establishment of *S. mutans* on teeth is dependent on the flora already present. Thus, the colo-

nization of *S. mutans* is inhibited on teeth already colonized by *A. viscosus* and *S. sanguis*, whereas on teeth harboring only one of these organisms *S. mutans* becomes established readily (34, 35). These findings are independent of the dietary concentration of sucrose. Furthermore, studies of human beings indicate that tooth surface sites infected with *S. mutans* may resist further infection by other *S. mutans* strains (22). In view of these observations, it seems important that the present studies involved plaque formation on cleaned tooth surfaces by indigenous organisms.

In contrast to *S. mutans*, all other species encountered colonized the teeth readily in the absence of sucrose, with casein or water as the only oral intake. In these experiments, *S. sanguis*, *S. mitior*, and *A. viscosus* constituted the major proportion of the bacteria recovered. The presence of sucrose did not enhance the relative affinity of any of these species for the tooth surface. Thus, the colonization pattern observed in the animal not infected with *S. mutans*, when provided with sucrose, differed in no way from the pattern observed in infected animals on casein or water (Table 1). These findings are consistent with the factors suggested to determine the capacity of *S. sanguis*, *S. mitior*, and *A. viscosus* to colonize nonretentive tooth surfaces (15; G. Rölla and M. Kilian, Caries Res., in press). The sucrose-independent colonization of a number of additional species, including haemophili, *A. actinomycetem-comitans*, neisseriae, and micrococci, is in accordance with the observed in vitro plaque formation by several examples of these species (Table 2). This in vitro plaque formation occurred independently of sucrose.

The present study has focused specifically on the initial phase of plaque formation on teeth. The subsequent increase in biomass is known to involve multiplication of the early colonizers as well as a continuous adsorption of bacteria from the environment (14). There is evidence to support the concept that the bacterial succession in developing dental plaque follows specific lines (37, 41) determined by the microenvironments existing on the dynamically changing plaque surface. Thus, plaque bacteria exhibit varying, but specific, degrees of affinity for each other in vitro (13, 23) and in vivo (21, 32), for extracellular polymers of microbial origin (12, 17, 33), and for salivary constituents (18). The pattern of the initial colonization is therefore likely to be of the utmost significance for the composition and pathogenic potential of mature plaque. In this context, and in view of the key role played by sucrose in the formation of dental caries (15), it is interesting that the only sucrose-provoked change that could be observed in early plaque formation in the present animal model was a significant increase in the proportion of *S. mutans* colonizing the teeth at the expense of *S. sanguis*. It is, therefore, tempting to postulate that the early sucrose-dependent establishment of *S. mutans* directly on the enamel pellicle plays a key role in the development of a cariogenic plaque. Knowledge of the unique ecological preferences of *S. mutans* may provide opportunities to inhibit its establishment.

ACKNOWLEDGMENTS

We gratefully acknowledge the stimulating discussions with D. Tustian. Jessie Bodenhoff (Statens Seriminstitut, Copenhagen) kindly confirmed the identity of some *Actinomyces* strains. Kirsten Holmgren is thanked for her skillful technique assistance.

LITERATURE CITED

1. **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.
2. **Bowen, W. H.** 1974. Effect of restricting oral intake to invert sugar or casein on the microbiology of plaque in *Macaca fascicularis* (Irus). Arch. Oral. Biol. 19:231-239.
3. **Bowen, W. H., and D. E. Cornick.** 1970. The microbiology of gingival-dental plaque. Recent findings from primate research. Int. Dent. J. 20:382-395.
4. **Carlsson, J.** 1967. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites of the oral cavity in man. Odont. Revy 18:55-74.
5. **Carlsson, J., H. Grahnén, G. Jonsson, and S. Wikner.** 1970. Establishment of *Streptococcus sanguis* in the mouths of infants. Arch. Oral Biol. 15:1143-1148.
6. **Colman, G., and R. E. O. Williams.** 1972. Taxonomy of some human viridans streptococci, p. 281-299. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.
7. **Cornick, D. E., and W. H. Bowen.** 1972. The effect of sorbitol on the microbiology of the dental plaque in monkeys (*Macaca irus*). Arch. Oral Biol. 17:1637-1648.
8. **Cowan, S. T., and K. J. Steel.** 1974. Manual for the identification of medical bacteria, 2nd ed. University Press, Cambridge.
9. **Cross, T., and M. Goodfellow.** 1973. Taxonomy and classification of actinomycetes, p. 11-112. *In* G. Sykes and F. A. Skinner (ed.), *Actinomycetales:* characteristics and practical importance. Academic Press Inc., New York.
10. **De Stoppelaan, J. D., J. van Houte, and O. B. Dirks.** 1970. The effect of carbohydrate restriction on the presence of *Streptococcus mutans*, *Streptococcus sanguis* and iodophilic polysaccharide-producing bacteria in human dental plaque. Caries Res. 4:114-123.
11. **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.
12. **Gibbons, R. J., and R. J. Fitzgerald.** 1969. Dextran-induced agglutination of *Streptococcus mutans*, and its potential role in the formation of microbial dental plaque. J. Bacteriol. 98:341-346.
13. **Gibbons, R. J., and M. Nygaard.** 1970. Interbacterial aggregation of plaque bacteria. Arch. Oral Biol. 15:1397-1400.

14. **Gibbons, R. J., and J. van Houte.** 1973. On the formation of dental plaques. J. Periodontol. 44:347-360.
15. **Gibbons, R. J., and J. van Houte.** 1975. Dental caries. Annu. Rev. Med. 26:121-136.
16. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. 29:19-44.
17. **Guggenheim, B., and H. E. Schroeder.** 1967. Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. Helv. Odont. Acta 11:131-152.
18. **Hillman, J. D., J. van Houte, and R. J. Gibbons.** 1970. Sorption of bacteria to human enamel powder. Arch. Oral Biol. 15:899-903.
19. **Hugh, R., and E. Leifson.** 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-26.
20. **Jensen, S. B., H. Löe, C. R. Schiøtt, and E. Theilade.** 1968. Experimental gingivitis in man. J. Periodont. Res. 3:284-293.
21. **Jones, S. J.** 1972. A special relationship between spherical and filamentous microorganisms in mature dental plaque. Arch. Oral Biol. 17:613-616.
22. **Jordan, H. V., H. R. Englander, W. O. Engler, and S. Kulczyk.** 1972. Observations on the implantation and transmission of *Streptococcus mutans* in humans. J. Dent. Res. 51:515-518.
23. **Kelstrup, J., and T. D. Funder-Nielsen.** 1974. Aggregation of oral streptococci with fusobacterium and actinomyces. J. Biol. Buccale 2:347-362.
24. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. J. Gen. Microbiol. 93:9-62.
25. **Kilian, M., and C. R. Schiøtt.** 1975. Haemophili and related bacteria in the human oral cavity. Arch. Oral Biol. 20:791-796.
26. **Krasse, B.** 1965. The effect of the diet on the implantation of caries-inducing streptococci in hamsters. Arch. Oral Biol. 10:215-221.
27. **Krasse, B., S. Edwardsson, I. Svensson, and L. Trell.** 1967. Implantation of caries-inducing streptococci in the human oral cavity. Arch. Oral Biol. 12:231-236.
28. **Larmas, M., K. K. Mäkinen, and A. Scheinin.** 1974. Turku sugar studies. III. An intermediate report on the effect of sucrose, fructose and xylitol diets on the numbers of salivary lactobacilli, candida and streptococci. Acta Odontol. Scand. 32:423-433.
29. **Lautrop, H.** 1960. Laboratory diagnosis of whooping-cough or *Bordetella* infections. Bull. W.H.O. 23:15-31.
30. **Liljemark, W. F., and R. J. Gibbons.** 1971. Ability of *Veillonella* and *Neisseria* species to attach to oral surfaces and their proportions present indigenously. Infect. Immun. 4:264-268.
31. **Liljemark, W. F., and R. J. Gibbons.** 1972. Proportional distribution and relative adherence of *Streptococcus mitior* (*mitis*) on various surfaces in the human oral cavity. Infect. Immun. 6:852-859.
32. **Listgarten, M. A., H. E. Mayo, and R. Tremblay.** 1975. Development of dental plaque on epoxy resin crowns in man. J. Periodontol. 46:10-26.
33. **McBride, B. C., and G. Bourgeau.** 1975. Dextran-induced aggregation of *Actinomyces viscosus*. Arch. Oral Biol. 20:837-842.
34. **Mikx, F. H. M., J. S. van der Hoeven, A. J. M. Plasschaert, and K. G. König.** 1975. Effect of *Actinomyces viscosus* on the establishment and symbiosis of *Streptococcus sanguis* in SPF rats on different sucrose diets. Caries Res. 9:1-20.
35. **Mikx, F. H. M., J. S. van der Hoeven, A. J. M. Plasschaert, and K. G. König.** 1976. Establishment and symbiosis of *Actinomyces viscosus*, *Streptococcus sanguis* and *Streptococcus mutans* in germ-free Osborne-Mendel rats. Caries Res. 10:123-132.
36. **Ørstavik, D., F. W. Kraus, and L. C. Henshaw.** 1974. In vitro attachment of streptococci to the tooth surface. Infect. Immun. 9:794-800.
37. **Ritz, H. L.** 1967. Microbial population shifts in developing human dental plaque. Arch. Oral Biol. 12:1561-1568.
38. **Saxton, C. A.** 1973. Scanning electron microscope study of the formation of dental plaque. Caries Res. 7:102-119.
39. **Syed, S. A., W. J. Loesche, H. L. Pape, Jr., and E. Grenier.** 1975. Predominant cultivable flora isolated from human root surface caries plaque. Infect. Immun. 11:727-731.
40. **Theilade, E., and J. Theilade.** 1970. Bacteriological and ultrastructural studies of developing dental plaque. p. 27-40. *In* W. D. McHugh (ed.), Dental plaque. E. and S. Livingstone Ltd., Edinburgh.
41. **Theilade, E., W. H. Wright, S. B. Jensen, and H. Löe.** 1966. Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. J. Periodont. Res. 1:1-13.
42. **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.
43. **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.