INFECTION AND IMMUNITY, Aug. 1979, p. 526-531
0019-9567/79/08-0526/06$02.00/0

Vol. 25, No. 2

Essential Dependence of Smooth Surface Caries on, and Augmentation of Fissure Caries by, Sucrose and *Streptococcus mutans* Infection

JASON M. TANZER

Departments of Oral Diagnosis and of Laboratory Medicine, University of Connecticut Health Center, Farmington, Connecticut 06032

Received for publication 15 May, 1979

Streptococcus mutans-free Osborne-Mendel rats were used to study the ability of well-characterized *S. mutans* strains of Bratthall serotypes *c*, *d*, and *E* to form plaque and cause caries when the animals consumed either sucrose- or glucose-containing diets. All of the serotype representatives successfully infected, colonized, and emerged in the oral ecology of animals, independent of the carbohydrate supplementation of the diet. However, the sucrose-containing diet supported higher percentages of *S. mutans* of all the serotypes in the plaque and greater amounts of plaque on the teeth. Smooth surface caries was essentially *S. mutans* dependent and sucrose dependent; fissure caries, although it was neither dependent on *S. mutans* infection nor sucrose consumption, was augmented by both. This sucrose-associated emergence of all three serotype representatives in the plaque flora and their virulence in the production of caries can be ascribed to their production of alkali-soluble α-(1 $\rightarrow$ 3)-rich glucans from sucrose.

The widely held notion that dental caries involving smooth surfaces of the teeth is sucrose dependent has recently come into question. It has been shown that the initial sorption of *Streptococcus mutans* to the teeth occurs in the absence of sucrose as well as in its presence (3). Also, the minimal colonizing dose of *S. mutans* for Sprague-Dawley rats is not dramatically different in the presence of sucrose- or of glucose-containing diets (35) when the infectant strain is a Bratthall serotype *c* (1) representative, although the minimal colonizing dose of *S. mutans* serotype *d* strains may be lower when rats are fed sucrose- than when they are fed glucose-containing diets (35). Furthermore, it is known that *S. mutans* can colonize the teeth of sucrase-deficient children who consume negligible amounts of sucrose (36) and of wild rats living in a relatively sucrose-free environment (4). Our group has recently shown that, whereas adherence in vitro of several serotype *a*, *b*, and *d* strains of *S. mutans* to tooth enamel and nichrome surfaces is supported by sucrose but not by glucose, serotype *c* and *E* strains colonize those surfaces with resultant good plaque growth in the presence of either sugar (34), although with perhaps less tenacity in the presence of glucose.

Abundant evidence indicates that serotype *c* cells of *S. mutans* are the ones most frequently isolated from human subjects in various parts of the world, with serotype *d* and *E* cells less frequently recovered, and serotype *a* and *b* cells only rarely recovered (2, 18, 28, 33). Nonetheless, the early experimental caries studies using rodents, in which the plaque-forming ability and cariogenicity of microorganisms were studied in the presence of sucrose or of other carbohydrate-containing diets, employed serotype *a*, *b*, or *d* cells and led to the conclusion that sucrose is by far the most cariogenic carbohydrate (7-9, 12, 15, 22, 24, 25). It is noteworthy that these serotypes are now known to be rather strictly sucrose dependent in their plaque formation, in contrast to serotype *c* and *E* cells (34).

It has thus become important to determine the in vivo plaque-forming ability of serotype *c* and *E* cells and to determine whether such cells induce comparable caries activity in vivo when used to infect animals consuming high glucose- as compared with high sucrose-containing diets. The present report demonstrates that serotype *c* and *E* representative strains, as well as serotype *d* strains, induce heavy plaque formation and are highly cariogenic in animals consuming a high sucrose diet but not in animals consuming a high glucose diet, although the successful colonization of animals by these strains is not dependent on sucrose consumption.

MATERIALS AND METHODS

Microorganisms. Spontaneously streptomycin-re-

sistant strains 6715-13 (Bratthall serotype *d*, 1,31), NCTC 10449S (Bratthall serotype *c*, 1,32), and LM7S (Bratthall serotype *E*, 1,13) were used. All strains were maintained in lyophile until ready for virulence test in experimental animals, at which time they were cultured in fluid thioglycolate medium containing 20% (vol/vol) meat extract (Difco Laboratories, Detroit, Mich.) and excess $CaCO_3$.

Animals, diets, and infection. Weanling Osborne-Mendel rats derived from specific-pathogen-free Osborn-Mendel (SPFOM) rats of the National Institutes of Health Animal Production Unit and free of indigenous *S. mutans* infection were used esentially under the conditions previously detailed (30–32). This animal strain was Caesarean-derived, suckled by SPFOM foster mothers, and specifically infected by four microorgainisms constituting normal indigenous rodent gut flora: two lactobacilli of unknown species, a strain of *S. faecalis*, and a strain of *Bacteroides* of unknown species (26). After weaning, the animals were provided caries test diet 2000 (23) containing either 56% sucrose or 56% glucose (Zeigler Brothers, Inc., Gardners, Pa.). The diet was supplied ad libitum, as was demineralized water. Three days after institution of this diet, each animal was orally infected with 0.2 ml of a fresh thioglycolate culture containing approximately 1.3×10^9 colony-forming units (CFU) of one of the three *S. mutans* strains studied per ml, or they remained uninfected. Thus, there were 12 groups with 8 animals in each group. Other data (not reported here) indicated that this infectious dose exceeds by at least two orders of magnitude the infectious dose required to establish colonization of these strains of *S. mutans* in all of the animals.

Recovery of microorganisms. The animals were cultured after being provided caries test diet and before bacterial challenge to monitor for possible extraneous *S. mutans* contamination. The recovery of infectants was tested generally 12 and 48 days after infectious challenge. The teeth were swabbed, and the swabs were placed into 0.05% yeast extract in 67 mM phosphate buffer (pH 7.0). The swab-containing tubes were immediately agitated in a Vortex mixer, and serial dilutions made in buffered yeast extract were spread on Mitis Salivarius agar with Chapman tellurite (MS; Difco), MS supplemented with 200 μg of streptomycin (MSS) per ml, and Trypticase soy agar supplemented with 5% sheep blood (B; BBL, Cockeysville, Md.). After incubation in candle jars, recoveries were recorded as the percentage of CFU on B which grew on MSS agar. Thus, the recoveries of streptomycin-labeled infectants with the morphology of *S. mutans* could be expressed in terms of total facultative flora. Also, the presence of non-streptomycin-resistant *S. mutans* infectants could be detected on MS agar. Logarithmic transformations of percent recoveries were carried out to improve the normalcy of distribution of data and to enable analysis of variance (29). The problems of sampling plaque from the teeth of small animals and of plate counts of streptococci (32) should be recoginized. The identites of recoverants were confirmed by well-established biochemical, physiological, and morphological techniques (28, 33).

Plaque and carious lesion evaluation. Upon sacrifice 50 days after infectious challenge, jaws were examined with plaque in situ and photographed. The jaws were defleshed by beetles, and carious enamel areas were scored by the method of Keyes (21). The so-called morsal category of carious lesions was plotted as a component of smooth surface caries, as recently indicated to be appropriate (L. A. Rinehimer and J. M. Tanzer, J. Dent. Res., in press). The experimental history of the jaws was unknown to the scorer. Differences among group caries score means were tested by analysis of variance.

RESULTS

There were no differences in animal weight gains during the course of the experiments, either between the animals infected by different microorganisms or between the animals consuming the glucose or sucrose diets.

Oral swabs of uninfected animals always failed to yield recovery of either streptomycin-resistant or streptomycin-sensitive *S. mutans*. Table 1 gives the percent recoveries of streptomycin-tagged *S. mutans* strains at 12 and 48 days postinfection. At 12 days postinfection, the recovery percentages of 6715-13 and of 10449S were much higher from sucrose-consuming than from glucose-consuming rats. Although a similar tendency was apparent for LM7S-infected rats,

TABLE 1. *Infectants recovered*[a]

Infectant	Day 12		Day 48	
	Glucose diet	Sucrose diet	Glucose diet	Sucrose diet
NCTC-10449S	1.9 ± 0.5	37.1 ± 10.2[b]	12.3 ± 3.9	46.9 ± 7.4[b]
LM7S	2.6 ± 1.2	7.4 ± 3.0[c]	5.4 ± 2.7	28.1 ± 2.8[b]
6715-13	0.9 ± 0.3	19.1 ± 6.9[b]	1.8 ± 1.2	53.0 ± 8.9[b]
Uninfected[d]	0.0	0.0	0.0	0.0

[a] Values indicate mean percent ± standard error of the mean.

[b] For comparison of infectant percent recoveries from sucrose-consuming rats to corresponding glucose-consuming rats, $P < 0.001$.

[c] For comparison of infectant percent recoveries from sucrose-consuming rats to corresponding glucose-consuming rats, $P > 0.05$.

[d] Uninfected animals yielded no recovery of either streptomycin-sensitive or streptomycin-resistant *S. mutans*.

the difference was not statistically significant at this time. At 48 days postinfection, all infected rat groups showed much higher recovery of the tagged *S. mutans* from sucrose-consuming than from glucose-consuming groups of animals. In no case were streptomycin-sensitive *S. mutans* recovered from any of these rats; therefore, there was no evidence of extraneous contamination of the animal groups or of the presence of an indigenous *S. mutans* infectant. Although there was a tendency for the percent recovery of the serotype *c* (10449S) and *E* (LM7S) representatives to be higher than that for the serotype *d* representative (6715-13) from animals consuming the glucose diet, this was statistically significant only in the case of 10449S ($P < 0.05$) at 48 days. The percent recovery of both 10449S and 6715-13 was significantly higher ($P < 0.05$) than that of LM7S at 48 days.

Before the animal jaws were cleaned, they were examined for plaque in situ and photographed. Rats infected by any of the three representatives of serotypes *c*, *d*, and *E* of *S. mutans* had more plaque on the teeth if they had been consuming the sucrose- rather than the glucose-containing diet (Fig. 1). In fact, jaws from infected animals consuming the glucose diet could not be distinguished on this basis from the jaws of animals uninfected by *S. mutans*.

Figure 2 illustrates the scores of carious enamel areas from all animal groups studied, and Table 2 summarizes the statistical comparisons of the data. *S. mutans*-infected animals consuming the sucrose-containing diet had higher caries scores than similarly infected ones consuming the glucose-containing diet. This difference was quantitatively most impressive with respect to smooth surface caries scores; glucose supported little smooth surface caries activity. Sucrose consumption also significantly augmented sulcal caries activity beyond the level supported by glucose diet consumption for the 10449S-infected animals.

As expected (30), infection by any of the three *S. mutans* serotype representatives greatly increased smooth surface caries activity by comparison with the uninfected control animals consuming the sucrose diet. The level of this increase was not quite statistically significant ($P < 0.05$) for LM7S-infected animals. However, this effect was not observed in animals consuming the glucose diet. Infection by all of the three serotype strain representatives augmented sulcal caries activity beyond the level caused by the indigenous flora of the rats if the animals had consumed either sucrose-containing or glucose-containing diets. This augmentation was just below the level of statistical significance for sucrose-consuming LM7S-infected rats and for glucose-consuming 6715-13-infected rats, but was otherwise statistically significant at the levels stipulated in Table 2. It was most impressive in the case of 10449S-infected rats.

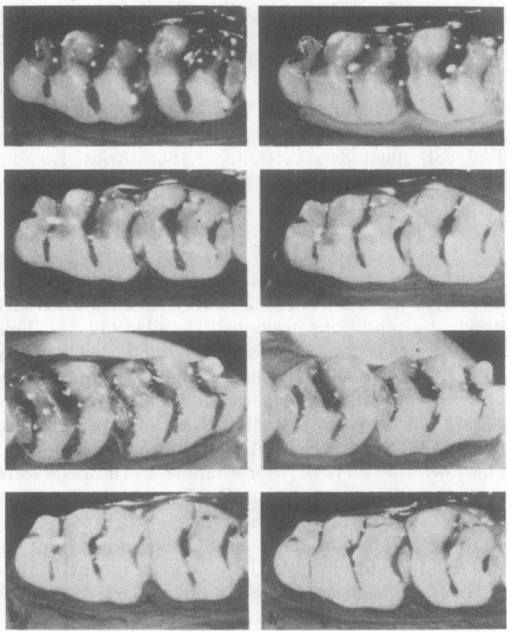

FIG. 1. *Linguo-occlusal view of typical safranine-stained mandibular first and second molars with plaque in situ. (a) NCTC-10449S, sucrose diet; (b) NCTC-10449S, glucose diet, (c) LM7S, sucrose diet; (d) LM7S, glucose diet; (e) 6715-13, sucrose diet; (f) 6715-13, glucose diet; (g) uninfected, sucrose diet; (h) uninfected, glucose diet. Note that food residues and impacted hairs, as well as microorganisms, stain in the tooth fissures.*

Among all rat groups not infected by *S. mutans*, consumption of the sucrose-containing diet supported no more sulcal caries activity than did consumption of the glucose-containing diet; however, among these same groups, sucrose consumption supported slightly more smooth surface caries activity in one experiment than did glucose consumption.

DISCUSSION

Early studies in experimental animals showed a strong sucrose dependency (7, 9, 12, 17, 22, 24, 39) of caries in hamsters, an animal model whose teeth are essentially free of fissures, thus possessing only smooth surfaces (19). These studies employed Bratthall serotype *a* and *b* strains such as HS-1, HS-6, E-49, AHT, 3720, and BHT. In rats, which possess both smooth and fissured tooth surfaces (20) like humans, the potent effect

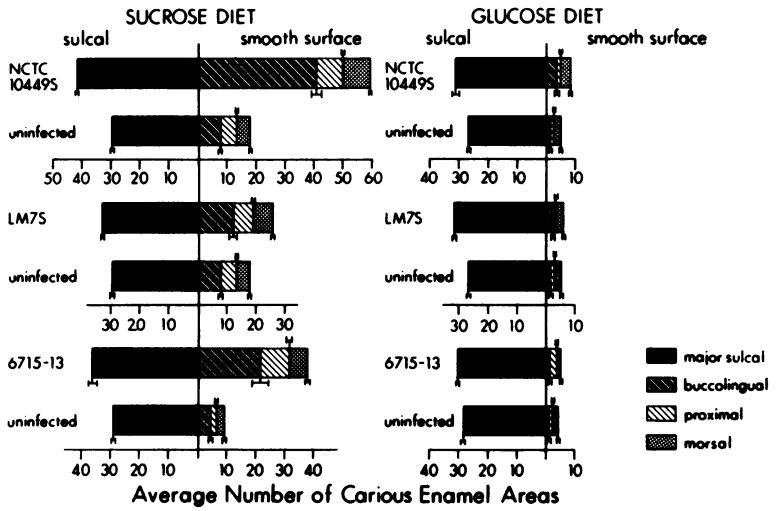

FIG. 2. *Average number of carious enamel areas (±standard error of the mean) of Osborne-Mendel rats consuming diet 2000 containing either 56% sucrose or glucose. Rats were infected by either S. mutans NCTC-10449S (serotype c), LM7S (serotype E), or 6715-13 (serotype d), or were left uninfected. There were 8 animals in each of the 12 groups.*

TABLE 2. *Statistical comparisons of caries scores, P values*[a]

Infectant strain	Infected vs. uninfected				Sucrose vs. glucose diet	
	Sucrose		Glucose		Sulcal	Smooth surface
	Sulcal	Smooth surface	Sulcal	Smooth surface		
NCTC-10449S	<0.0001	<0.0001	<0.05	NS	<0.0001	<0.0001
LM7S	NS	NS	<0.05	NS	NS	<0.005
6715-13	<0.05	<0.0005	NS	NS	NS	<0.0001
Uninfected	—	—	—	—	NS[b]	<0.05[b]
					NS[c]	NS[c]

[a] NS, Not statistically significant ($P > 0.05$).
[b] For 10449S and LM7S experiment.
[c] For 6715-13 experiment.

of sucrose by comparison with other carbohydrates is also well known (8, 12, 25) and, indeed, evidence of the potency of sucrose in caries is well known from human diet studies (27).

Unfortunately, most studies of rat caries which tested the effects of diet depended upon the undefined indigenous flora of the animal, which at least sometimes contained *S. mutans* contaminants of the animals populations used. In many cases, the nature of the flora was not of concern to the investigators, and serotyping of *S. mutans* was not then available. Restrospective evaluation of those few older reports of the carbohydrate relationship to caries where *S. mutans* was isolated from rats (8, 15, 25) and where there was subsequent study of the serological and genetic identities of these strains (1, 5) has revealed them to be of Bratthall serotypes *a*, *b*, and *d*. Furthermore, in those cases where rats, otherwise free of *S. mutans*, were specifically infected by an *S. mutans* strain, usually Bratthall serotype *d* representatives (such as strains SL-1 OMZ-176, 6715, and KIR) were used (7, 15, 16, 25, 30–32).

However, in recent years, it has become clear that serotype *a* and *b* strains are rare and that serotype *c* is the most common among humans, with serotypes *d* and *E* being less common than serotype *c*. The demonstration that: (i) *S. mutans* serotype *c* and *E* strains form plaque on surfaces in vitro in the presence of glucose, albeit less tenaciously than in the presence of sucrose (34); (ii) adsorption of *S. mutans* strain 6715 (serotype *d*) to either enamel or to saliva-coated hydroxyapatite occurs as well for cells exposed to sucrose as for cells exposed to glucose (3); (iii)

humans (36) and rats (4) consuming little sucrose are colonized by *S. mutans*; and (iv) rats may be infected by serotype *c* and *E* strains readily when consuming glucose diets (35) has indicated the need to reexamine the caries conduciveness of glucose and sucrose as a function of the serotype of *S. mutans* infectant. To this end, the present data demonstrate that sucrose-containing diet 2000 is much more supportive of dental caries than glucose-substituted diet 2000 in animals infected by representatives of the most common serotypes, *c*, *d*, and *E*, of *S. mutans*, consistent with the reports of the rare serotypes *a* and *b*, and the common serotype *d*, reviewed above. This effect, although most evident at the smooth surface sites of teeth, is also evidenced to some degree in the fissures (sulci) of teeth. The data support the concept that smooth surface caries is essentially *S. mutans* dependent and sucrose dependent; fissure caries, although neither *S. mutans* dependent nor sucrose dependent, is augmented in activity by both.

The present study also indicates that both glucose and sucrose diets support infection, successful colonization, and ecological emergence of serotype *c*, *d*, and *E* strains as substantial components of the recoverable tooth flora, consistent with the report of van Houte et al. (35). Also, consistent with the findings of that group (35, 37, 38), it indicates that generally the percentage of the plaque flora comprised by *S. mutans* becomes higher in sucrose-consuming than in glucose-consuming rats, for all serotypes tested. It extends the fingings of those studies to indicate that these effects are associated with decidely higher virulence on both smooth and fissure tooth surfaces. This potent sucrose effect is presumably related to the synthesis of cell-associated alkali-soluble glucans rich in α-(1 $\rightarrow$ 3) linkages previously detailed by our group to be required for tenacious plaque formation and virulence of serotype *d* strain 6715-13 (10, 11, 30) and to be associated with tenacious plaque formation in the presence of sucrose, but not in the presence of glucose, by serotype *c* strain NCTC-10449S and serotype *E* strain LM7S (34).

Caution should be exercised that the present data not be overinterpreted to conclude that serotype *c* strains are more virulent than *d* strains and, in turn, *E* strains. To test the question of comparative virulence of serotypes, multiple fresh isolates of each must be simultaneously studied.

It should be noted that these data suggest that among the indigenous non-*S. mutans* flora of these rats there exists some microorganism(s) whose caries activity is slightly augmented by exposure to sucrose by comparison with exposure to glucose, although this effect is not nearly so dramatic as the smooth surface cariogenic effect of *S. mutans* strains in the presence of sucrose.

ACKNOWLEDGMENTS

I thank L. A. Rinehimer, F. N. Woodiel, and V. Y. Pavlakis for able technical assistance.

This study was supported by Public Health Service grant DE-03758 from the National Institute of Dental Research.

LITERATURE CITED

1. **Bratthall, D.** 1970. Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans*. Odontol. Revy **21**:143-152.
2. **Bratthall, D.** 1972. Demonstration of *Streptococcus mutans* strains in some selected areas of the world. Odontol. Revy **23**:1-10.
3. **Clark, W. B., and R. J. Gibbons.** 1977. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. Infect. Immun. **18**:514-523.
4. **Coykendall, A., D. Bratthall, K. O'Connor, and R. A. Dvarskas.** 1976. Serological and genetic examination of some nontypical *Streptococcus mutans* strains. Infect. Immun. **14**:667-670.
5. **Coykendall, A. L., O. P. Daily, M. J. Kramer, and M. E. Beath.** 1971. DNA-DNA hybridization studies of *Streptococcus mutans*. J. Dent. Res. **50**:1131-1139.
6. **Edwardsson, S.** 1968. Characteristics of human caries-inducing streptococci resembling *Streptococcus mutans*. Arch. Oral Biol. **13**:637-646.
7. **Fitzgerald, R. J., and H. V. Jordan.** 1968. Polysaccharide producing bacteria and caries, p. 79-86. *In* R. S. Harris, (ed.), Art and science of dental caries research. Academic Press Inc., N.Y.
8. **Fitzgerald, R. J., H. V. Jordan, and H. R. Stanley.** 1960. Experimental caries and gingival pathologic changes in the gnotobiotic rat. J. Dent. Res. **39**:923-935.
9. **Fitzgerald, R. J., and P. H. Keyes.** 1960. Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J. Am. Dent. Assoc. **61**:9-19.
10. **Freedman, M. L., D. Birkhed, and K. Granath.** 1978. Analyses of glucans from cariogenic and mutant *Streptococcus mutans*. Infect. Immun. **21**:17-27.
11. **Freedman, M. L., and J. M. Tanzer.** 1974. Dissociation of plaque formation from glucan-induced agglutination in mutants of *Streptococcus mutans*. Infect. Immun. **10**:189-196.
12. **Frostell, G., P. H. Keyes, and R. H. Larson.** 1967. Effect of various sugars and sugar substitutes on dental caries in hamsters and rats. J. Nutr. **93**:65-76.
13. **Gibbons, R. J., K. S. Berman, P. Knoettner, and B. Kapsimalis.** 1966. Dental caries and alveolar bone loss in gnotobiotic rats infected with capsule-forming streptococci of human origin. Arch. Oral Biol. **11**:549-560.
14. **Grenby, T. H.** 1963. The effects of some carbohydrates on experimental dental caries in the rat. Arch. Oral Biol. **8**:27-30.
15. **Guggenheim, B., K. G. König, E. Herzog, and H. R. Muhlemann.** 1966. The cariogenicity of different carbohydrates tested in rats in relative gnotobiosis with a *Streptococcus* producing extracellular polysaccharide. Helv. Odontol. Acta **10**:101-113.
16. **Guggenheim, B., K. G. König, and H. R. Muhlemann.** 1965. Modifications of the oral bacterial flora and their influence on dental caries in the rat. I. The effects of

inoculating four labeled strains of streptococci. Helv. Odontol. Acta **9**:121-129.

17. **Gustafson, G., E. Stelling, E. Abramson, and E. Brunius.** 1959. Experimental dental caries in golden hamsters. IX. The effect of a cariogenic diet fed at intervals. Arch. Oral Biol. **1**:42-47.
18. **Hamada, A., N. Masuda, T. Ooshima, S. Sobue, and S. Kotani.** 1976. Epidemiological survey of *Streptococcus mutans* among Japanese children. Identification and serological typing of the isolated strains. Jpn. J. Microbiol. **20**:33-44.
19. **Keyes, P. H.** 1944. A method of recording and scoring gross carious lesions in the molar teeth of Syrian hamsters. J. Dent. Res. **23**:439-444.
20. **Keyes, P. H.** 1958. Dental caries in the molar teeth of rats. I. Distribution of lesions induced by high-carbohydrate low-fat diets. J. Dent. Res. **37**:1077-1087.
21. **Keyes, P. H.** 1958. Dental caries in the molar teeth of rats. II. A method for diagnosing and scoring several types of lesions simultaneously. J. Dent. Res. **37**:1088-1099.
22. **Keyes, P. H., and R. J. Fitzgerald.** 1962. Dental caries in the Syrian hamster. IX. Arch. Oral Biol. **7**:267-277.
23. **Keyes, P. H., and H. V. Jordan.** 1964. Periodontal lesions in the syrian hamster. III. Findings related to an infectious and transmissible component. Arch. Oral Biol. **9**:337-400.
24. **Krasse, B.** 1965. The effect of caries-inducing streptococci in hamsters fed diets with sucrose or glucose. Arch. Oral Biol. **10**:223-226.
25. **Larje, O., and R. H. Larson.** 1970. Reduction of dental caries in rats by intermittent feeding with sucrose substitutes. Arch. Oral Biol. **15**:805-816.
26. **Schaedler, R. W., R. Dubos, and R. Costello.** 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. **122**:59-66.
27. **Scheinen, A., K. K. Makinen, and K. Ylitalo.** 1975. Turku sugar studies. I. An intermediate report on the effect of sucrose, fructose and xylitol diets on the caries incidence in man. Acta Odontol. Scand. **33**(Suppl. 70): 5-34.
28. **Shklair, I. L., and H. J. Keene.** 1976. Biochemical characterization and distribution of *Streptococcus mutans* in three diverse populations, p. 201-210. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Microbial aspects of dental caries. Information Retrieval Ltd., Washington, D.C.
29. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods, p. 593. Iowa State University Press, Ames, Iowa.
30. **Tanzer, J. M., M. L. Freedman, R. J. Fitzgerald, and R. H. Larson.** 1974. Altered virulence of mutants of *Streptococcus mutans* defective in polysaccharide synthesis. Infect. Immun. **10**:197-203.
31. **Tanzer, J. M., M. L. Freedman, F. N. Woodiel, R. L. Eifert, and L. A. Rinehimer.** 1976. Association of *Streptococcus mutans* virulence with synthesis of intracellular polysaccharide, p. 597-616. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Microbial aspects of dental caries. Information Retrieval Ltd., Washington, D.C.
32. **Tanzer, J. M., G. J. Hageage, Jr., and R. H. Larson.** 1973. Variable experiences in immunization of rats against *Streptococcus mutans*-associated dental caries. Arch. Oral Biol. **18**:1425-1439.
33. **Thomson, L. A., W. Little, and G. J. Hageage, Jr.** 1976. Application of fluorescent antibody methods in the analysis of plaque samples. J. Dent. Res. **55**:A80-A86.
34. **Tinanoff, N., J. M. Tanzer, and M. L. Freedman.** 1978. In vitro colonization of *Streptococcus mutans* on enamel. Infect. Immun. **21**:1010-1019.
35. **van Houte, J. H., R. C. Burgess, and H. Onose.** 1976. Oral implantation of human strains of *Streptococcus mutans* in rats fed sucrose or glucose diets. Arch. Oral Biol. **21**:561-564.
36. **van Houte, J. H., and S. Duchin.** 1975. *Streptococcus mutans* in the mouths of children with congenital sucrase deficiency. Arch. Oral Biol. **20**:771-773.
37. **van Houte, J. H., and V. N. Upeslacis.** 1976. Studies of the mechanism of sucrose-associated colonization of *Streptococcus mutans* on teeth of conventional rats. J. Dent. Res. **55**:216-222.
38. **van Houte, J. H., V. N. Upeslacis, H. V. Jordan, Z. Skobe, and D. B. Green.** 1976. Role of sucrose in colonization of *Streptococcus mutans* in conventional Sprague-Dawley rats. J. Dent. Res. **55**:202-215.
39. **Zinner, D. D., J. M. Jablon, A. P. Aran, and M. S. Saslaw.** 1965. Experimental caries induced in animals by streptococci of human origin. Proc. Soc. Exp. Biol. Med. **118**:766-770.