INFECTION AND IMMUNITY, Dec. 1981, p. 662-675
0019-9567/81/120662-14$02.00/0

Vol. 34, No. 3

Sucrose-Induced Ecological Response of Experimental Dental Plaques from Caries-Free and Caries-Susceptible Human Volunteers

GLENN E. MINAH,* GISELLE B. LOVEKIN, AND JAMES P. FINNEY

Departments of Microbiology and Pediatric Dentistry, Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore, Baltimore, Maryland 21201

Received 6 April 1981/Accepted 21 July 1981

Microbial succession, experimental cariogenicity, and sucrose metabolism were examined in dental plaques which developed on sterile bovine enamel inserts in acrylic palatal appliances. The appliances were worn for a period of 14 days by 10 caries-free and 10 caries-susceptible human volunteers. Three of six enamel inserts on each appliance were exposed extraorally to 10% sucrose in 0.85% saline six times a day, and three were exposed simultaneously to 0.85% saline as a control environment. The responses of the plaques to the high-sucrose environment in both caries status populations were compared. In all plaques, exposure to 10% sucrose stimulated the succession of *Veillonella* spp., *Lactobacillus* spp., *Streptococcus salivarius*, and, to a lesser extent, *Streptococcus mutans* and a decline in levels of *Streptococcus sanguis*, *Neisseria* spp., and gram-negative anaerobic rods. Plaques from caries-free mouths, in contrast to those from caries-susceptible mouths, harbored higher levels of *Veillonella* spp., gram-negative anaerobic rods, and *Neisseria* spp. and lower levels of *Lactobacillus* spp. Sucrose-exposed plaques from caries-free mouths also induced less enamel microhardness changes and formed less lactic acid from [^{14}C]sucrose during a 60-min incubation at 37°C than did comparable plaques from caries-susceptible mouths. The experiments revealed consistent differences in the ecological response to a cariogenic substrate environment in plaques from the two populations, with plaques from caries-free subjects exhibiting less cariogenic potential than those from caries-susceptible subjects.

Numerous theories to explain apparent resistance to dental caries in humans have been proposed (for review, see reference 45), including the possibility that the oral microflora in such individuals differs from that caries-susceptible (CS) individuals. Although it has been widely reported that a caries-associated dental plaque harbors higher concentrations of *Streptococcus mutans* (3, 9, 17, 21, 36, 37, 44) or lactobacilli (2-4, 16, 22) and lower concentrations of *Streptococcus sanguis* (7, 21, 22) than plaque on non-diseased dental surfaces, only a few cross-sectional cultural studies have pursued the question of oral microbial differences in individuals who do and do not experience dental caries. Two such investigations revealed higher concentrations of lactobacilli in mouths of CS subjects than in mouths of caries-resistant (CR) subjects (15, 38). More recently, it was found that naval recruits with no history of caries experience exhibited lower levels of *S. mutans* in molar fissures than did recruits with past caries experience (18, 36). Another group of investigators, however, reported no difference in plaque *S. mutans* levels or in levels of five serotypes of *S. mutans* in the two types of individuals (39).

An objective of this investigation was to examine the oral microflora of individuals with no history of caries experience longitudinally during a controlled exposure to a cariogenic substrate environment. The ecological responses of the oral bacteria as expressed by microbial population changes and certain metabolic activities might reveal unique characteristics of this population as compared with CS individuals.

To adequately control the substrate environment and maximize experimental manipulation of plaques, a removable appliance model system was employed. This appliance, which was similar to one described by Ostrom and Koulourides (28), contained enamel surfaces on which plaque could develop intraorally. The model system permitted simultaneous exposure of the experimental plaques to test (cariogenic) and control (non-cariogenic) substrates extraorally and an assessment of plaque cariogenicity by sequential microhardness measurements of the associated enamel. With the use of this device, the popu-

lation shifts of predominant or important bacteria in plaque caused by frequent exposure to sucrose, plaque-induced experimental cariogenicity, and in vitro metabolic response of the plaques to sucrose were evaluated and compared.

MATERIALS AND METHODS

RDM. The removable dental model (RDM) was a horseshoe-shaped palatal appliance made of acrylic with ring clasps for maxillary second molars (Fig. 1). Stainless steel rings (0.180-in. [ca. 4.5-mm] outer diameter; 0.015-in. [ca. 0.4-mm] wall; 3-mm height; Small Parts, Inc.) were embedded in the distal flanges of the appliance (three on each side). Smaller stainless steel cylinders (0.148-in. [ca. 3.7-mm] outer diameter; 0.015-in. wall; 5-mm height) containing polished bovine enamel disks were placed into the rings in the appliance. The disks were formed from polished bovine enamel cubes (4 mm by 4 mm by 3 mm; cut with a Micro-Matic Precision Wafering Machine, model WMSA-2178). These were forced into the stainless steel cylinders by using no. 110 pliers. The resulting arrangement provided a 2-mm-deep well over the enamel surface. Acrylic covers (1 to 2 mm thick on stainless steel springs (0.002 in. [ca. 0.1 mm]) were positioned over the wells. The covers provided a stagnation area where plaque could develop, reduced salivary exposure of the experimental plaques, and thereby the potential for contamination of the plaques by tongue and salivary microflora, and prevented irritation to the tongue by the protruding inserts. As the covers fit loosely against the tops of the cylinders inoculation of the wells by the oral bacteria occurred readily. The covers also enabled a relatively constant volume of plaque to be collected in each well (Fig. 2). This appliance was a modified version of the Ostrom and Koulourides ICT model (28), which is a mandibular crozat appliance containing bovine enamel slabs covered with dacron gauze on the buccal flanges. Human enamel, which would have been the ideal material for this type of experiment, was avoided due to the concern that teeth from different humans might contain different levels of fluoroapatite and have different enamel microhardness values (30). The bovine enamel, which resembles human enamel in mineral content and microhardness (11), was acquired from two ani-

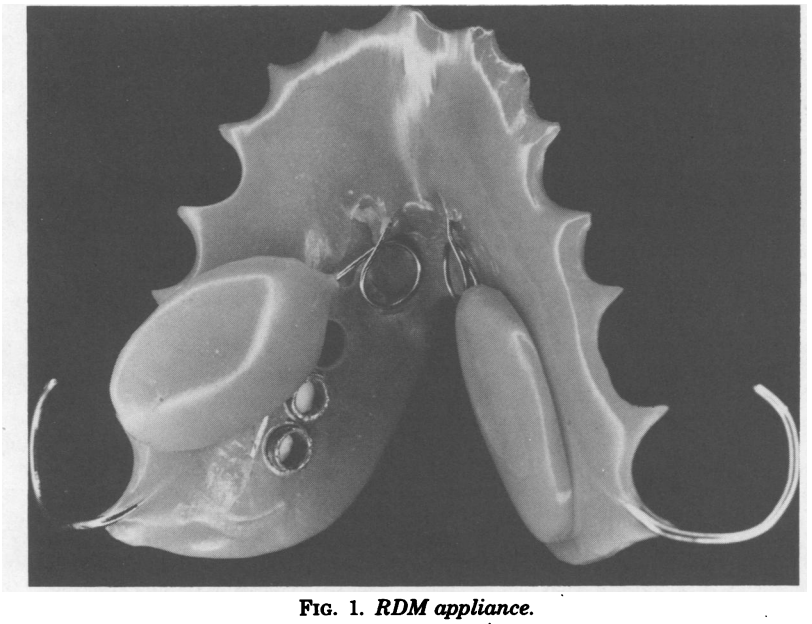

FIG. 1. *RDM appliance.*

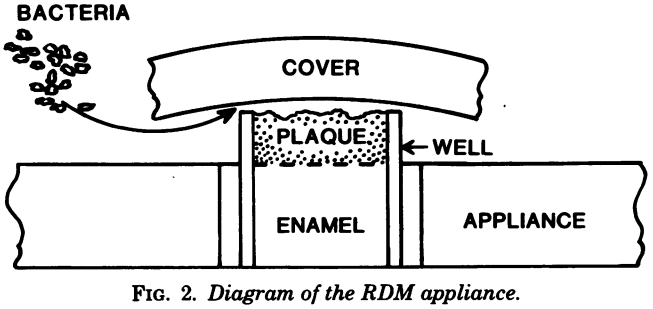

FIG. 2. *Diagram of the RDM appliance.*

mals from the same herd and feed lot and exhibited equivalent microhardness values (see below).

Experimental design. Twenty dental and graduate students at the Baltimore College of Dental Surgery, Dental School of the University of Maryland at Baltimore wore the RDM for a period of 14 days. Of the 20 participants, one withdrew from the experiment after 1 week due to an inability to tolerate the appliance. Three of the subjects wore the appliance on two occasions separated by at least one year. The data from these subjects were processed separately. Ten volunteers had no history of dental caries in the primary or permanent dentitions (CF group). The remaining volunteers were susceptible to caries as evidenced by dental restorations (CS group). CS subjects exhibited a range of 8 to 20 and a mean of 12 restored dental surfaces. The median age of the volunteers was 22 years. The age range was 19 to 38 years. The participants wore the appliance at all times except during the ingestion of food or beverages other than water and during immersion of the appliance in the experimental substrates. Each appliance was immersed six times a day for a period of 20 to 30 min each in a 10% sucrose solution in normal saline (test solution) and normal saline (control solution). Half of the inserts were immersed in the test solution, and half were immersed in the control solution simultaneously. At 7 and 14 days one stainless steel cylinder containing the plaque and enamel insert was removed from each side of the appliance for culturing (see below) and microhardness determinations of the enamel associated with the plaques (see below). Test and control plaques in the third wells were employed in another investigation.

An unstimulated saliva specimen from each subject was collected at the same 7- and 14-day time periods for culturing to monitor the effect of the appliance on the salivary flora.

Finally, sucrose metabolism of the 14-day test and control plaques of eight CF and eight CS subjects was evaluated. Quantities of lactic acid, cell-bound and insoluble products, soluble polysaccharides, CO_2, and total volatile products (excluding CO_2) produced in test and control plaques after exposure to [^{14}C]sucrose were determined and compared (see below).

These experiments were designed to allow correlations between plaque microbial composition, enamel microhardness changes, and quantity of particular metabolic products formed to be made. Comparison between test and control plaques in each mouth and between plaques of CF and CS subjects were made and statistically evaluated (see below).

Microbiological procedures. The enamel inserts were placed individually in 1 ml of reduced transport fluid (20) and introduced with the saliva specimens into a Coy anaerobic chamber (1). The plaque was dislodged from the inserts and dispersed by sonication for 10 s (Kontes cell disruptor), serially diluted in reduced transport fluid, and inoculated on the selective and nonselective solid media listed below with a Spiral Systems spiral plater. The media were as follows.

(i) MM10 anaerobic agar. Total colony counts on nonselective MM10 sucrose blood agar (MM10 anaerobic agar) (20), which was incubated for 7 days at 37°C in the anaerobic chamber, served as the total viable count of the specimen. Recognizable colony types on MM10 anaerobic agar were of *S. mutans, S. sanguis, Streptococcus salivarius*, and black-pigmented *Bacteroides* species. Each unidentified colony appearing on this media was Gram stained. Counts of identifiable colonies or unidentified colonies (Gram stains) on this medium and colony counts of selective media were expressed as percent occurrence relative to the total vialbe count.

(ii) MM10 CO_2 agar. MM10 CO_2 agar, which was incubated for 7 days at 37°C in candle jars, allowed the quantitation of total air-susceptible facultative bacteria. The total viable counts on MM10 CO_2 and MM10 anaerobic agars were expressed as the CO_2/anaerobic ratio.

(iii) MM10 aerobic agar. MM10 aerobic agar, which was incubated for 7 days at 37°C aerobically, allowed determination of aerobic and facultative total viable counts and, with the MM10 anaerobic count, the aerobic/anaerobic ratio.

(iv) Mitis salivarius bacitracin agar. Mitis salivarius bacitracin agar (5, 14), which was incubated for 3 days anaerobically at 37°C and 1 day aerobically at room temperature, was selective for all *S. mutans* serotypes except B.

(v) Rogosa L agar. Total *Veillonella* spp. counts and catalase-positive *Veillonella* spp. counts were made on rogosa L agar (33). Liberation of bubbles when 3% H_2O_2 was added to the colony indicated positive catalase activity. Plates were incubated anaerobically for 4 days at 37°C.

(vi) Rogosa SL agar. Total *Lactobacillus* spp. counts were made on rogosa SL agar (35) after incubation in candle jars for 3 days at 37°C.

(vii) Neisseria agar. Neisseria agar (31) was selective for *Neisseria* spp. after aerobic incubation for 3 days at 37°C. Catalase and oxidase activities of each colony were determined. (*p*-Aminodimethylaniline from a moistened Taxo N disc [BBL Microbiology Systems] containing this reagent was applied to each colony; a darkened colony indicated a positive reaction.)

(viii) Columbia CNA agar. Columbia CNA agar (10), which was incubated for 3 days in candle jars at 37°C, selected for *Actinomyces*-like organisms. Catalase activity of each colony was determined.

(ix) MM10-kanamycin sulfate agar. MM10 agar containing 50 μg of kanamycin sulfate per ml (A. Syed Salam, personal communication) was selective for gram-negative anaerobic rods. It was incubated anaerobically for 7 days at 37°C.

(x) Sabouraud dextrose agar. Yeast colonies were quantitated on Sabouraud dextrose agar (13; BBL) after incubation in candle jars for 3 days at 37°C.

To avoid enumeration of nonspecific colonies on the selective agar, Gram staining of representatives of each colony type on each selective plate was performed. Frequently occurring nonspecific colonies were of streptococci and yeasts on rogosa SL agar, gram-negative anaerobic rods on rogosa L agar, *S. mutans* and *S. sanguis* on MM10 kanamycin agar, and streptococci and non-*Actinomyces*, gram-positive branched rods on CNA agar.

Although a more comprehensive microbiological analysis was desirable it would have been unfeasible in an investigation with such a large number of specimens. The procedures employed allowed the enumeration and proportional recovery of most of the important oral microorganisms at either the genus or species level to be made in a relatively rapid and simplified manner.

Enamel microhardness measurements. Enamel microhardness was measured by a diamond point indentation method as described by Koulourides and Volker (19) with a Tukon microhardness tester (model M) loaded with 500 g. The mean length of three indentations in enamel before it was exposed to the human mouths was converted into the Knoop hardness number (KHN) which represented the baseline value. The KHN of enamel after plaque exposure was similarly determined. The change in enamel hardness after exposure to the plaque was expressed as percent decalcification using the formula, percent enamel hardness change = [(KHN before oral exposure − KHN after oral exposure) × 100]/(KHN before oral exposure).

Metabolic procedures. Samples (100 μl) of the dispersed 14-day test and control plaques were introduced separately into 1-dram (ca. 3.7-ml) screw-capped vials containing 0.088% sucrose in reduced transport fluid. Uniformly labeled [^{14}C]sucrose (New England Nuclear Corp., 2 μCi in 50 μl of reduced transport fluid) was added (final sucrose concentration, 0.05%), and the mixture was incubated anaerobically for 60 min at 37°C in the anaerobic chamber. Sucrose utilization, lactic acid formation, soluble polysaccharide formation, production of insoluble and cell-bound products, and formation of CO_2 and total volatile products (excluding CO_2) were measured after 60 min of incubation by methods previously described (25, 26).

The metabolic products were quantitated and recorded as nanomoles of sucrose equivalents per 2×10^8 particles in the incubation mixture. Particle counts were performed with a Petroff-Hausser counting chamber in a Leitz microscope with a dark-field condenser. The data were expressed as normalized quantities and proportions of products formed.

Statistical analyses. The following statistical analyses were performed with data from CF, CS, and combined CF and CS specimens: (i) test-versus-control differences in microbiological values at 7 and 14 days (paired *t* test); (ii) test-versus-control differences in enamel microhardness changes at 7 and 14 days (paired *t* test); (iii) differences in test plaques, in control plaques, and in saliva of each individual at 7 and 14 days (paired *t* test); (iv) test-versus-control differences in metabolic values (quantity and proportion of products formed) at 14 days (paired *t* test); (v) microbiological, enamel microhardness changes, and metabolic differences between CF and CS subjects at either 7 and 14 days or 14 days (unpaired *t* test); (vi) correlations between plaque concentration of a microbial value and enamel microhardness change (correlation test); and (vii) correlation between quantity or proportion of lactic acid formed and enamel microhardness change (correlation test).

Statistical tests were performed on a Texas Instruments programmable prompting calculator (model SR-60). Differences consistent at the $P < 0.05$ level of confidence were considered to be significant. Differences consistent at a lower confidence level, $0.05 < P < 0.1$, were reported in an effort to reveal potential tendencies of the data.

RESULTS

Microbial differences in sucrose- versus saline-exposed plaques. As sucrose exposure represented the only variable affecting test and control plaques on each appliance, differences in microbial population shifts were attributed to this variable (Tables 1 and 2). In combined CF and CS data, total *Veillonella* spp. and *S. salivarius* concentrations were significantly higher in test plaques, and *S. sanguis* levels were significantly higher in control plaques. These differences were found at both 7 and 14 days. Total *Lactobacillus* spp. and gram-positive unbranched rods (presumed to be lactobacilli) reached significantly higher levels in test plaques after 14 days.

With a lower level of significance ($0.05 < P < 0.1$) as an indicator of potential population shifts, *S. mutans* and yeast concentration increased in the sucrose environment, whereas total *Neisseria* spp., gram-negative rods (selective media), and black-pigmented *Bacteroides* spp. decreased. Although many other microbial values varied considerably in test and control plaques, no other differences achieved significance.

Microbial succession in sucrose- or saline-exposed experimental plaques. In either the sucrose or saline substrate environment consistent microbial population shifts were observed between 7 and 14 days of the experimental period (Tables 1 and 2). In sucrose-exposed plaques (combined CF and CS subjects) gram-positive unbranched rods increased significantly, whereas total *Neisseria* spp., gram-negative cocci, and *S. sanguis* decreased significantly. *S. mutans* and total *Lactobacillus* spp. increased in these plaques, but not significantly ($0.05 < P < 0.1$). In saline-exposed plaques, total *Neisseria* spp., gram-positive branched rods (presumed to be *Actinomyces*-like bacteria), and gram-negative rods (nonselective media) were significantly higher at 7 versus 14 days. *S. sanguis* levels also decreased between 7 and 14 days ($0.05 < P < 0.1$). Other proportional shifts occurred in each environment, but were not significantly different between 7 and 14 days.

Microbial differences between CF and CS subjects. Each microbial category in plaques of the two populations was analyzed by a comparison of (i) proportional values in test versus control substrate environments, (ii) longitudinal shifts in either the sucrose or saline environ-

TABLE 1. *Proportions of bacterial isolates in 7-day experimental dental plaques from CF and CS subjects*

Bacterial isolate	Sucrose-exposed CF	Saline-exposed CF	Sucrose-exposed CS	Saline-exposed CS	Sucrose-exposed total	Saline-exposed total
Selective media						
S. mutans	0.05[a] (±0.12)[b]	0.05 (±0.05)	0.01 (±0.02)	0.03 (±0.06)	0.03 (±0.07)	0.04 (±0.06)
Lactobacillus spp.	0.02 (±0.37)[c]	0.0001 (±0.0002)	5.99 (±(14.5)	0.38 (±0.11)	3.41 (±10.99)	0.02 (±0.09)
Veillonella spp. (total)	6.99 (±6.16)[d]	2.27 (±2.3)	6.26 (±2.66)[c]	2.66 (±2.95)	6.52 (±6.29)[e]	2.5 (±2.63)
Veillonella spp. (catalase positive)	0.42 (±0.48)	0.39 (±0.75)	0.60 (±0.96)	0.52 (±0.88)	0.52 (±0.77)	0.46 (±0.81)
Neisseria spp.	1.78 (±1.98)[c]	14.02 (17.75)	0.89 (±1.68)[d]	7.40 (±9.9)	1.13 (±1.83)[c]	9.88 (±13.23)
Gram-negative rods	2.03 (±3.06)[c]	4.81 (±5.05)	2.22 (±2.23)	3.12 (±3.95)	2.13 (±2.57)[c]	3.91 (±4.44)
Black-pigmented *Bacteroides* spp.	0.0	0.34 (±7.2)	0.0	0.02 (±0.06)	0.0[c]	0.17 (±0.05)
Actinomyces-like (total)	6.79 (±5.26)[d]	1.7 (±2.33)	3.88 (±5.34)	4.11 (±2.64)	5.09 (±5.41)	3.0 (±2.71)
Actinomyces-like (catalase positive)	1.26 (±2.59)	0.75 (±0.83)	1.85 (±3.3)	1.78 (±1.79)	1.58 (±2.88)	1.3 (±1.48)
Nonselective media						
Gram-positive cocci	45.6 (±12.8)[c]	45.5 (±17.4)	44.99 (±14.57)	45.47 (16.23)	46.37 (±15.18)	45.48 (±16.09)
Gram-negative cocci	15.8 (±9.83)[d]	13.78 (±8.98)	18.78 (±11.93)	16.09 (±12.24)	17.55 (±12.05)[c]	15.23 (±10.86)
Gram-positive branched rods	26.5 (±9.59)	28.12 (±13.3)	21.15 (±15.05)[c]	30.1 (±16.43)	23.53 (±12.85)	29.04 (±14.79)
Gram-negative unbranched rods	2.65 (±6.7)	0.0	9.21 (±19.1)[c]	0.0	6.29 (±14.9)[c]	0.0
Gram-negative rods	4.01 (±3.04)	3.57 (±3.45)	1.5 (±3.3)	3.0 (±3.89)	2.61 (±3.35)	3.34 (±3.54)
S. sanguis	1.21 (±1.31)[d]	4.61 (±4.12)	1.05 (±1.56)[c]	4.27 (±6.7)	1.12 (±1.4)[e]	4.4 (±5.63)
S. mutans	0.11 (±0.032)	0.2 (±0.49)	0.74 (±2.11)	0.0	0.44 (±1.6)	0.08 (±0.31)
S. salivarius	5.59 (±4.76)[c]	1.08 (±4.76)	8.68 (±12.08)	4.14 (±7.29)	7.30 (±9.53)[d]	2.88 (±5.71)
Yeasts	1.36 (±2.7)	0.73 (±1.8)	0.34 (±1.1)	0.42 (±1.26)	0.87 (±2.07)	0.53 (±1.43)
Aerobic/anaerobic ratio	0.68 (±0.1)[d]	0.63 (±0.08)	0.71 (±0.61)	0.57 (±0.39)	0.70 (±0.45)	0.59 (±0.33)
CO_2/anaerobic ratio	0.94 (±0.88)	0.69 (±0.3)	0.62 (±0.33)	0.57 (±0.39)	0.75 (±0.61)	0.62 (±0.35)

[a] Value expressed as % total viable count om MM10 anaerobic agar.
[b] Standard deviation.
[c] Test-versus-control difference significant at $0.05 < P < 0.1$ (paired t test).
[d] Test-versus-control difference significant at $P < 0.05$ (paired t test).
[e] Test-versus-control difference significant at $P < 0.001$ (paired t test).

TABLE 2. *Proportions of bacterial isolates in 14-day experimental dental plaques from CF and CS subjects*

Bacterial isolate	Sucrose-exposed CF	Saline-exposed CF	Sucrose-exposed CS	Saline-exposed CS	Sucrose-exposed total	Saline-exposed total
Selective media:						
S. mutans	1.6[a] (±3.63)[b]	0.02 (±0.03)	1.4 (±3.9)[c]	0.06 (±0.08)	1.49 (±3.6)[c]	0.04 (±0.06)
Lactobacillus spp.	7.95 (±13.4)[c]	0.004 (±0.01)	15.41 (±29.3)[c]	4.46 (±12.3)	11.9 (±22.9)[d]	2.38 (±9.03)
Veillonella spp. (total)	13.36 (±8.49)[d]	2.89 (±3.23)	4.37 (±5.3)	4.21 (±2.81)	8.56 (±8.15)[d]	3.59 (±4.47)
Veillonella spp. (catalase positive)	1.97 (±3.96)	0.43 (±0.40)	0.56 (±1.05)	0.35 (±0.58)	1.16 (±2.67)	0.39 (±0.53)
Neisseria spp.	3.34 (±6.12)[d]	9.34 (±6.88)	2.43 (±4.39)[c]	2.79 (±2.37)	2.85 (±5.1)[c]	5.85 (±5.88)
Gram-negative rods	8.4 (±11.39)	6.89 (±6.87)	1.49 (±1.84)	2.21 (±1.95)	5.59 (±8.76)	4.55 (±5.4)
Black-pigmented *Bacteroides* spp.	0.0	0.14 (±0.38)	0.0	0.08)±0.13)	0.0[c]	0.09 (±0.25)
Actinomyces-like (total)	12.57 (±19.32)	2.49 (±0.82)	4.61 (±2.15)	2.89 (±5.02)	9.03 (±14.6)	2.66 (±3.13)
Actinomyces-like (catalase positive)	0.054 (±0.11)[c]	0.36 (±0.4)	1.53 (±1.82)	1.33 (±2.38)	0.71 (±1.36)	0.79 (±1.58)
Nonselective media						
Gram-positive cocci	41.21 (±17.06)	46.86 (±13.97)	30.78 (±16.02)[d]	40.63 (±16.17)	36.4 (±16.79)[c]	45.5 (±15.48)
Gram-negative cocci	20.54 (±18.75)	17.86 (±6.77)	8.65 (±5.97)	15.4 (±7.32)	15.05 (±15.12)	15.68 (±7.16)
Gram-positive branched rods	26.5 (±11.98)	17.86 (±10.87)	26.92 (±21.59)	22.9 (±11.23)	26.71 (±16.65)	19.69 (±10.77)
Gram-positive unbranched rods	6.97 (±9.77)[c]	0.0	31 (±30.6)[d]	7.4 (±15.11)	19.06 (±23.96)[e]	2.85 (±10.26)
Gram-negative rods	5.53 (±3.97)	7.26 (±6.45)	3.27 (±5.05)	8.0 (±7.83)	4.49 (±4.46)[c]	7.52 (±6.82)
S. sanguis	0.64 (±0.74)	2.37 (±3.0)	0.18 (±0.35)[c]	1.28 (±1.37)	0.4 (±0.59)[d]	1.79 (±2.26)
S. mutans	0.0	0.0	4.13 (±8.18)	0.33 (±1.0)	2.2 (±6.17)	0.2 (±0.8)
S. salivarius	6.33 (±7.2)[c]	1.83 (±4.1)	5.5 (±6.23)[d]	1.26 (±2.19)	5.89 (±6.46)[d]	1.55 (±3.15)
Yeasts	1.27 (±2.27)	0.33 (±0.87)	0.43 (±1.06)	0.4 (±0.82)	0.85 (±1.74)[c]	0.33 (±0.81)
Aaerobic/anaerobic ratio	0.37 (±0.27)	0.48 (±0.08)	0.52 (±0.24)	0.39 (±0.31)	0.44 (±0.25)	0.43 (±0.23)
Co_2/anaerobic ratio	0.67 (±0.58)	0.48 (±0.11)	0.73 (±0.27)	0.48 (±0.31)	0.70 (±0.44)	0.48 (±0.24)

[a,b,c,d,e] See footnotes to Table 1.

ments or the saliva between 7 and 14 days, and (iii) plaque levels of each microbial value at a particular time period or in a particular substrate (CF versus CS subjects).

When test-versus-control plaque microbial differences were compared, more pronounced population shifts were found at 7 days in CF subjects (Table 1) and at 14 days in CS subjects (Table 2). After 7 days, total *Veillonella* spp., total *Actinomyces*-like bacteria, gram-negative cocci, and *S. salivarius* were significantly higher, and *S. sanguis* was significantly lower in test plaques of CF individuals. Within the same time period only total *Neisseria* spp. were significantly higher in control plaques of CS individuals.

After 14 days, total *Veillonella* spp. were significantly higher and total *Neisseria* spp. were significantly lower in test plaques of CF subjects. In CS subjects, gram-positive unbranched rods and *S. salivarius* were significantly higher, and gram-positive cocci were significantly lower in test plaques.

Within a particular substrate environment most of the significant population changes occurred in the CS plaques. Only *S. sanguis* levels, which decreased, varied significantly between 7 and 14 days in CF subjects in the sucrose environment. In the same substrate environment, gram-positive unbranched rods of CS subjects increased significantly, whereas *S. sanguis* and gram-negative cocci decreased significantly between 7 and 14 days.

Within the saline environment the only significant longitudinal variation in CF subjects was in plaque levels of *Actinomyces*-like bacteria which increased. In CS subjects the CO_2/anaerobic ratio increased, gram-positive cocci decreased, gram-positive branched rods decreased, and *Actinomyces*-like bacteria decreased significantly between 7 and 14 days.

Salivary concentrations of the microbial categories in CS subjects did not change at either the $P < 0.05$ or the $0.05 < P < 0.1$ level of significance between days 7 and 14 of the experimental period. In CF subjects, however, gram-positive branched rods increased significantly. Additionally, levels of total *Lactobacillus* spp., total *Veillonella* spp., and catalase-positive *Veillonella* spp. and the CO_2/anaerobic ratio increased in these subjects, but at a lower level of significance ($0.05 < P < 0.1$).

A direct comparison between CF and CS subjects revealed that most of the significant population differences occurred in the sucrose-exposed plaques (Table 3). CF subjects harbored significantly higher levels of gram-negative rods (nonselective media) at 7 days and higher total *Veillonella* spp. at 14 days than did CS subjects.

TABLE 3. *Comparative differences between experimental plaques of CF and CS subjects*

Plaque age (days)	Sucrose-exposed plaques	Saline-exposed plaques
7	Gram-negative rods on nonselective media, $P < 0.05$,[a] CF > CS[b]	Aerobic/anaerobic ratio, $0.05 < P < 0.1$, CF > CS Gram-negative cocci, $0.05 < P < 0.1$, CS > CF Gram-positive branched rods, $0.05 < P < 0.1$, CS > CF
14	*Veillonella* spp., $P < 0.05$, CF > CS Gram-negative rods on selective media, $0.05 < P < 0.1$, CF > CS Gram-positive cocci, $0.05 < P < 0.1$, CF > CS Gram-negative cocci, $0.05 < P < 0.1$, CF > CS *S. sanguis*, $0.05 < P < 0.1$, CF > CS Gram-positive unbranched rods, $P < 0.05$, CS > CF	*Neisseria* spp. $P < 0.05$, CF > CS Gram-negative cocci, $0.05 < P < 0.1$, CF > CS Gram-negative rods, $0.05 < P < 0.1$, CS > CF

[a] Significance of difference (*t* test).

[b] CF > CS denotes higher proportions in CF versus CS plaques.

Gram-positive unbranched rods were significantly higher in CS test plaques at 14 days. In saline-exposed CF plaques, total *Neisseria* spp. were significantly higher at 14 days.

The concentrations of gram-negative cocci, gram-positive branched and unbranched rods, gram-positive cocci, and *S. sanguis* and the aerobic/anaerobic ratios in CF and CS plaques were also notably different, but the differences did not achieve significance.

Enamel microhardness changes. Sucrose-exposed plaques were associated with considerably greater microhardness changes of the enamel inserts than were the saline-exposed plaques (Table 4). The test-versus-control differences were highly significant at 7 and 14 days in both populations. The CS 14-day test plaques decalcified the associated enamel to a significantly greater extent than did their CF counterparts. The CS test plaques produced a mean of 76.49% microhardness change compared with 58.73% in CS subjects. Differences between CF and CS subjects at 7 days in sucrose- or saline-exposed enamel or at 14 days in saline-exposed enamel were not significant.

Correlation between enamel microhardness change and plaque composition. The microbial values which showed a significant neg-

ative correlation with enamel microhardness were *Veillonella* spp. in all test plaques at 7 days and in CF test plaques at 14 days and gram-positive unbranched rods in CS plaques at 14 days. There were no significant positive correlations observed.

In CF and CS combined control plaques at 14 days there were significant correlations between *Veillonella* spp. levels and between *S. mutans* levels and enamel decalcification. When the Knoop hardness number of test enamel inserts showing the most extensive microhardness and those showing the least microhardness change were viewed in relation to test and control *S. mutans* and *Lactobacillus* levels in the plaques, the plaque harboring the highest levels of *S. mutans* was associated with the most enamel microhardness change, followed by plaques harboring high levels of *Lactobacillus* spp. (Table 5). Noticeable succession of *S. mutans* appeared in plaques which harbored no detectable *Lactobacillus* spp., whereas decline or disappearance of *S. mutans* appeared in plaques associated with high levels of *Lactobacillus* spp. Plaques exhibiting small or no test versus control differences in levels of *S. mutans* or *Lactobacillus* spp. showed only slight enamel decalcification.

Metabolic differences in experimental plaques. Sucrose metabolism by test versus control plaques was analyzed in an effort to further differentiate CF and CS experimental plaques (Tables 6 and 7).

Quantitatively, there were no significant dif-

TABLE 4. *Percent enamel microhardness change after exposure to experimental dental plaques*

RDM plaque type	% Enamel microhardness change[a] on day 7	P^b	% Enamel microhardness change on day 14	P^b
CF test	48 (±25.37)[c]	<0.001	58.73 (±19.86)	<0.015
CF control	0.0 (±0.0)		30 (±25.92)	
CS test	48.55 (±25.15)		76.49 (±19.8)	<0.001
CS control	0.0 (±0.0)	<0.001	25.82 (±19.51)	
Total test	48.25 (±24.81)		66.19 (±27.12)	<0.001
Total control	0.0 (0.0)	<0.001	27.79 (±22.11)	
CS versus CF test		NS[d]		<0.05
CS versus CF control		NS		NS

[a] Percent enamel microhardness change was determined as described in the text.

[b] Test-versus-control enamel microhardness differences were tested by the paired *t* test, and CF-versus-CS enamel microhardness differences were tested by the *t* test.

[c] Standard deviation.

[d] NS, Not significant.

TABLE 5. *Plaque levels of S. mutans and lactobacilli in sucrose- and saline-exposed plaques in relation to sucrose-exposed enamel microhardness change*

Caries type	% Enamel microhardness decrease[a]	% *S. mutans*[b]		% *Lactobacillus* spp.[c]		Plaque age (days)
		Sucrose exposed	Saline exposed	Sucrose exposed	Saline exposed	
CS	99	11.0[b]	0.0003	0	0	14
CS	91	0	0.18	41	0.001	14
CS	91	0	0	79	0.001	14
CS	82	0.1	0.002	0	0	7
CF	79	0.001	0.03	29	0.008	14
CF	74	0.6	0.04	0	0	14
CS	73	0.034	0.2	0.19	0.02	7
CF	67	0.0002	0.12	0.8	0.0005	7
CS	61	0.06	0.15	0.5	0.003	14
CF	55	0.1	0.09	0	0	7
CS	41	0.003	0.001	0.001	0.0005	14
CF	18	0	0	0.007	0	14
CF	18	0.009	0	N.D.	N.D.	14
CF	10	0.001	0	N.D.	0	7
CS	10	0.014	0.015	0.003	0.0001	7
CS	2	0.0004	0.06	0.00005	0	7

[a] Percent enamel microhardness decrease was determined as described in the text.

[b] Expressed as (colony count on mitis salivarius bacitracin agar/colony count on MM10 anaerobic agar) × 100.

[c] Expressed as (colony counts on rogosa SL agar/colony counts on MM10 anaerobic agar) × 100. N.D., Not done.

TABLE 6. *By-products of sucrose metabolism formed in 14-day experimental dental plaque in CF and CS subjects (quantitative values)*

Caries type	Plaque exposure	nmol of [^{14}C]sucrose equivalents/2 × 10^8 particles/60-min incubation					
		Sucrose utilized	Lactic acid	Cell-bound and insoluble products	Soluble polysaccharide	Volatile products (except CO_2)	CO_2
CF	Sucrose	91.72[a] (±93.94)[b]	18.68 (±13.68)	26.91 (±26.76)	12.4 (±8.3)	31.5 (±32.42)	1.23 (±1.72)
	Saline	118.36 (±120.11)	18.96 (±17.21)	45.4 (±57.34)	9.08 (±8.3)	40.85 (±42.89)	2.07 (±2.11)
CS	Sucrose	71.77 (±82.13)[c]	28.6 (±37.13)	16.02 (±20.37)	3.8 (±2.14)	22.22 (±25.33)[d]	0.58 (±0.41)[d]
	Saline	85.48 (±87.8)	20.89 (±18.68)	18.25 (±24.23)	9.5 (±16.03)	35.66 (±38.97)	2.09 (±2.57)
Total	Sucrose	82.95 (±85.34)	19.97 (±28.89)	20.96 (±23.17)	5.05 (±3.6)	26.28 (±28.02)	0.87 (±1.17)
	Saline	102.9 (±105.59)	20.04 (±17.48)	30.13 (±42.68)	7.7 (±13.06)	37.93 (±39.41)	2.31 (±2.08)

[a,b,c,d] See footnotes *a* through *d* of Table 1.

TABLE 7. *By-products of sucrose metabolism formed in 14-day experimental dental plaques from CF and CS subjects (proportional values)*

Caries type	Plaque exposure	% of [^{14}C]sucrose consumed/2 × 10^8 particles/60-min incubation				
		Lactic acid	Cell-bound and insoluble products	Soluble polysaccharide	Volatile products (except CO_2)	CO_2
CF	Sucrose	25.7[a] (±13.1)[b]	20.2 (±11.5)	20.7 (±26.7)[c]	32 (±19.6)	1.36 (±1.09)[c]
	Saline	21.8 (±13.6)	25.8 (±14.2)	13.9 (±18.3)	41.3 (±21.8)	1.9 (±1.08)
CS	Sucrose	41.5 (±16.2)[d]	20.4 (±7.9)	8.49 (±4.16)	21.8 (±9.97)[e]	1.39 (±1.24)[e]
	Saline	28.7 (±14.8)	20.6 (±9.0)	10.3 (±8.56)	37.1 (±13.2)	2.66 (±2.48)
Total	Sucrose	34.6 (±16.52)[d]	20.35 (±9.27)	14.37 (±18.79)	28.12 (±15.69)[e]	1.38 (±1.14)[e]
	Saline	25.7 (±14.3)	22.88 (±11.43)	9.83 (±7.44)	40.1 (±16.8)	2.33 (±1.98)

[a,b,c,d,e] See footnotes *a*, *b*, *c*, *e*, and *d*, respectively, of Table 1.

ferences in the rates of sucrose consumption by test and control plaques in CF or CS subjects or combined subjects. The saline-exposed plaques of each group showed higher mean values for sucrose consumption rates than did sucrose-exposed plaques.

Lactic acid formation by all test and control plaques did not differ significantly in mean quantitative values, but CS test plaques transformed significantly more of the consumed sucrose into lactic acid than did controls. This did not occur in CF plaques.

Proportionally, more soluble polysaccharide was formed by CF test plaques than controls ($0.05 < P < 0.1$). Otherwise, there were no significant test-versus-control differences in cell-bound and insoluble product or soluble polysaccharide formation in both caries status groups.

Saline-exposed plaques of CS subjects formed significantly more CO_2 and other volatile products, both quantitatively and proportionally, than did sucrose-exposed plaques. This was not found in CF plaques.

DISCUSSION

Validity of the removable model system. Partial characterization of the microflora which colonized either extracted molar fissures worn in a palatal appliance (43), molar fissure segments worn in excavated molar teeth (41, 42), mylar fissure-like inserts in excavated molars (36), and dacron gauze-covered bovine enamel slabs worn in mandibular crozat appliances (29) have been performed. Although none of these investigations nor the present investigation found a conspicuous absence of a predominant plaque species or the colonization of the experimental surfaces by bacteria which were not indigenous to the oral cavity, there were variations in the mean proportional recovery of certain types of bacteria. For example, *S. salivarius* as a proportion of total plaque streptococci after 7 days reportedly did not colonize fissure segments worn in a palatal appliance (43), comprised 15% in natural fissure segments worn in excavated molars (42), comprised 40 to 70% in dacron gauze on enamel slabs in a mandibular crozat appliance (29), and reached 16% in sucrose-exposed plaques and 6.4% in saline-exposed plaques in the present investigation. Such interexperiment variations might be the result of the particular environments created by the removable surfaces in situ. The nature of the site configuration, surface material, intraoral location, and substrate environment which differed among the studies cited above theoretically selected for bacteria best

suited to thrive there. Evidence in favor of this argument was obtained in this laboratory when the RDM appliance was tested with the enamel inserts in a configuration which simulated a molar fissural econiche (G. E. Minah, G. C. Lovekin, and L. Chen, *Proceedings of the Fourth Annual Workshop/Conference on Foods, Nutrition and Dental Health*, in press). Sucrose-exposed plaques from these sites contained notably higher mean concentrations of *S. mutans*, *S. sanguis*, and *Veillonella* spp. after 10 days than RDM test plaques at 7 or 14 days.

Of importance is the question of similarities between natural and artificial plaques. Such a comparison is difficult due not only to the wide microbial population variations which occur from site to site within the same mouth, but also to an inability to control many variables which affect plaque composition on natural teeth.

A comparison in this laboratory of natural plaques from dental students and caries-active children (Minah et al., in press) with experimental plaques showed general similarities in proportion of gram-positive and -negative cocci, gram-positive branched rods, and gram-negative rods as well as general similarities in concentrations of certain bacteria on selective media such as *Neisseria* spp. and *Veillonella* spp. and gram-negative anaerobic rods. The most noticeable differences were in levels of *S. sanguis*, which were higher in fissures and smooth surfaces of dental students; the levels of *S. mutans*, which were higher in fissures of dental students and caries-active children; and the levels of lactobacilli, which were higher in sucrose-exposed experimental plaques. The interproximal plaques from caries-active children most closely resembled sucrose-exposed experimental plaques.

Clearly, the experimental plaques in this investigation did not resemble the saliva or tongue microflora. This fact is supported by significant differences (paired *t* test) observed in levels of *S. mutans*, *Veillonella* spp., catalase-positive *Actinomyces*-like bacteria, *S. salivarius*, and gram-positive branched rods and the CO_2/anaerobic ratio between saliva at either 7 or 14 days and comparable control experimental plaques removed from CF and CS subjects. An influence of the appliance on the salivary flora, on the other hand, was evident and presents a potential limitation of this type of experiment.

From the evidence discussed, the study of plaques on removable surfaces should be considered as a helpful adjunct to, but not a substitute for, investigations of natural human plaque. The increased experimental versatility of the model ideally would introduce concepts which could then be applied to natural model systems.

Cultural findings. Many of the microbial categories found to be affected by the sucrose environment in the present investigation were similarly affected in natural plaque during either controlled high- and low-sucrose dietary regimes or during radiation-induced cariogenesis. High-sucrose diets were associated with a proportional ascension of *S. mutans* (6, 7, 12) and lactobacilli (6) in plaque and a decline in *S. sanguis* levels (6, 7). A follow-up low-sugar diet in the same volunteers caused a reversal of these population shifts (6, 7, 12). In pooled plaque from xerostomic patients, *S. mutans*, lactobacilli, and yeasts proportionally increased after radiological alteration of the salivary glands, whereas *S. sanguis*, *Neisseria* spp., *Bacteroides* spp., and *Fusobacterium* spp. levels decreased (4).

A definitive explanation for the population shifts observed in the present study and in natural plaque studies would require currently unavailable knowledge of ecological selective factors and microbial interactions. Oral microbial ecosystems are further complicated by the fact that the inhabitants can result from both internal bacterial growth and acquisition from other sources, oral or extraoral. However, much evidence, including the present findings, indicates that plaques do undergo internal microbial succession (23, 29), a phenomenon which has been linked to the pathogenic potential of the ecosystem.

Although most of the bacterial categories affected by sucrose exposure were similar in CF and CS subjects, differences existed both in the extent of the population shift and in the number of bacterial categories affected during a particular time period or longitudinally in a particular substrate environment.

It is unclear why CF plaques showed stronger test-versus-control differences at 7 days than CS plaques, or why the longitudinal shifts in either the sucrose or saline environments occurred to a greater extent in CS plaque. It is also unclear why certain components of the salivary microflora changed significantly between 7 and 14 days in the CF subjects, but not the CS subjects. The validity of and explanation for these characteristics require further research.

The apparent tendency for CF plaques to harbor higher levels of *Veillonella* spp., *Neisseria* spp., *S. sanguis*, and gram-negative anaerobic rods, none of which is believed to be cariogenic (4, 6, 7, 21, 22, 24, 27, 40, 44), and for CS plaques to harbor more lactobacilli, a suspected cariogenic group (2, 4, 16, 22), supports the hypothesis that the microflora in CF mouths might not shift to a cariogenic profile as readily in a cariogenic dietary environment as that in CS mouths.

The enamel microhardness changes which

were considerably greater in test plaques of CS subjects also supported this hypothesis. The correlations between enamel microhardness changes and lactobacilli in CS test plaques adds further support, but the correlation between enamel microhardness changes and levels of total *Veillonella* spp. conflicts with this hypothesis.

At least one previous study has depicted this organism as being potentially anticariogenic. Dual infections of *Veillonella alcalescens* and *S. mutans* in gnotobiotic rodents induced fewer dental caries than did monoinfections of *S. mutans* (24). Its positive correlation with decalcification in the present study, though, gives *Veillonella* spp. a cariogenic image.

The ability of *Veillonella* spp. to thrive on and transform lactic acid to less-dissociated acetic and propionic acids has been proposed to be an anti-cariogenic capability (32), but neither the exact timing and nature of these metabolic reactions nor the effect of acetic and propionic acids on the teeth in vivo is adequately understood. The sharp decrease in concentrations of this organism between 7 and 14 days in CS test plaques which also were associated with the highest degree of enamel decalcification found suggests that this organism does not tolerate a highly acidic environment. This argument concurs with a previous in vitro study of *Veillonella* spp. in environments of various pH (33).

In light of the reduced level of decalcification in CF test enamel and increased levels of *Veillonella* spp. in the associated plaques versus comparable CS plaques, the hypothesis that the *Veillonella* spp. protected the CF enamel can be considered. From another viewpoint, the CF plaques might not have reached the level of plaque acidity where *Veillonella* spp. would decline and enamel decalcification would increase. This question should be addressed by future research.

A correlation between plaque lactobacillus levels and enamel decalcification parallels similar findings of longitudinal investigations of natural plaque (2, 4, 16, 22). The failure of *S. mutans* levels to coincide with extent of enamel decalcification, however, appears to conflict with several natural plaque studies (3, 9, 17, 21, 36, 37, 44). It is possible that this organism requires a period of time longer than 14 days to emerge in high concentrations. Long-term studies of fissure segments implanted in molar restorations revealed plaque *S. mutans* concentrations higher than those found in fissure segments which were implanted for a short period of time (41, 42).

When the data were evaluated individually with subjects listed according to extent of enamel decalcification, at least three noteworthy tendencies emerged. First, relatively high plaque levels (greater than 10% of the total viable count) of either *S. mutans* or total lactobacilli were associated with the most extensive enamel microhardness change. Whether these organisms were directly responsible for the enamel effects is unclear since high levels of other acidogenic bacteria were present in these plaques. Second, there might have been an anatagonistic relationship between lactobacilli and *S. mutans*. Perhaps *S. mutans* could not compete at pH environments which favored succession of lactobacilli. Other types of antagonistic relationships, however, should not be ruled out. The third and final tendency was the apparent resistance to sucrose-induced microbial population changes in some individuals. These plaques, interestingly, were associated with the least enamel decalcification. The reasons for the apparent stability of some plaque ecosystems are not known, but are being pursued in this laboratory.

The enamel decalcification produced by control plaques which theoretically did not contact a cariogenic substrate deserves comment. Whether the control plaques were exposed to residual carbohydrates which were retained in the subjects' mouths after eating, or plaques of considerable volume (13.15 mm^3 in the stainless steel wells) can induce a decalcifying effect after an extended period of time, is presently not clear. The fact that *S. mutans* and *Veillonella* spp. levels correlated significantly with enamel decalcification introduces the possibility that microbial shifts contributed to this occurrence.

Metabolic findings. If the test plaques were assumed to be more cariogenic than the controls, their failure to utilize sucrose and form lactic acid at a faster rate than control plaques contrasts with metabolic patterns of caries-associated natural plaques which utilize sucrose at a faster rate and produce more lactic acid than do plaques from noncarious sites of the same teeth (26). Although the control plaques on the appliances roughly resembled natural plaques from noncarious surfaces in microbial composition, test plaques harbored a notably different microflora than did plaques from diseased surfaces, particularly in the proportions of *S. mutans* (carious plaques, 30.1%; RDM 14-day test plaques, 1.5%). As rates of glycolysis of pure cultures of *S. mutans* were shown to exceed those of other major plaque inhabitants (27), this fact might explain the differences in question. Plaques from non-cariogenic sites in the natural plaque study did not produce volatile acids and CO_2 quantitatively to a greater extent than did cariogenic plaques, but did proportionally, supporting a similar finding of CS plaques in the present investigation (Table 7).

Although an analysis of metabolic capabilities

of each plaque component would be required for a definitive explanation of the test-versus-control differences in volatile product formation in CS plaques, higher combined mean levels of gram-negative cocci, gram-positive branched rods, and gram-negative rods existed in the control plaques. These bacteria usually produce more volatile metabolic products from sucrose than do oral gram-positive cocci and gram-positive unbranched rods (lactobacilli), the predominant inhabitants of test plaques (27).

The CF test plaques, on the other hand, harbored high levels of *Veillonella* spp. at 14 days, which might have been responsible for the less-pronounced test-versus-control differences in volatile products in these plaques. These species form mainly acetic and propionic acids and CO_2 from lactate, a by-product of plaque fermentation.

The highly significant proportional test-versus-control difference in lactic acid production in CS plaques suits the depiction of these plaques as being cariogenic. Further, these plaques were the only type which showed a significantly positive correlation between quantity and proportion of lactic acid formed and extent of enamel decalcification. Another experiment conducted with 10 (6 CS and 4 CF) of the 16 subjects in the present investigation (G. E. Minah, M. Matheus, and J. P. Finney, Arch. Oral Biol., in press) suggested that the pH optimum of glycolytic enzymes might be a distinguishing characteristic of sucrose- and saline-exposed plaque. In reaction mixtures of lower pH (6.0 and 5.0), test plaques utilized sucrose and formed lactic acid at significantly faster rates than did controls. In vitro studies with pure cultures have also shown that an acidic pH increased the glycolytic activity of cariogenic bacteria compared with levels in a neutral pH environment (S. Harper, Ph.D. dissertation, University of Michigan, Ann Arbor, 1980).

Summary of CF and CS plaque characteristics. The experiments discussed in this paper showed clear differences between experimental plaques of CF and CS subjects, despite the fact that the populations were relatively small.

The sucrose-exposed plaques of CF subjects contained higher levels of the types of bacteria and the ecological responses which generally fit a non-cariogenic profile, with the exception of large numbers of *Veillonella* spp. The latter fact, as discussed above, might also be an indication of low cariogenicity. CF test plaques, additionally, were not able to decalcify the associated enamel to the extent found in CS enamel and did not produce lactic acid to the extent that CS plaques did. CS plaques harbored a microflora more characteristic of a cariogenic ecosystem with fewer *Neisseria* spp. and gram-negative anaerobic rods than in CF plaques and higher concentrations of lactobacilli. The CS plaques, in addition, showed more pronounced population shifts during sucrose exposure, a fact which might be indicative of cariogenic potential.

An explanation of differences between the two populations can only be speculated about, but at least two general viewpoints can be adopted.

According to one, CF subjects possessed oral factors which controlled the ecology of the artificial ecosystems or which limited the caries activity by the plaques. The fact that the plaques developed in an environment which could supply these factors is in accordance with this hypothesis. Should such a hypothesis be valid, the conceptualization of and therapeutic approach to dental caries would be affected.

A second viewpoint would hold that the ecological environments of CF mouths selected for bacteria which were non- or even anti-cariogenic. Such ecosystems might not be able to respond rapidly to a short-term exposure to sucrose, but under prolonged sucrose exposure could shift to a state analogous to CS test plaques. An obvious question in this regard is that of frequency of sucrose consumption in CF and CS individuals. If CF individuals did consume sucrose less frequently their mouths might harbor a microflora which possessed non-cariogenic attributes both in composition and ecological responses to sucrose exposure.

One diet survey did, in fact, find CF subjects to consume significantly less sucrose than CS subjects (8).

Diet analysis, being an unreliable experimental tool, may not conclusively differentiate the two populations, but two investigations (46, 47) found that the sweet taste threshold was significantly lower in CF versus CS subjects, implying that they might not consume the quantity of "sweet-tasting food" that CS individuals do. This evidence supports the second viewpoint discussed above.

Whether CF subjects possess oral anti-cariogenic defense mechanism or harbor a sucrose-starved microflora is presently not clear. An in-depth pursuit of this question might reveal important facts about dental caries.

ACKNOWLEDGMENT

This project was supported by Public Health Service grant DE 04795 from the National Institute of Dental Research.

LITERATURE CITED

1. **Aranki, A., S. A. Syed, E. B. Kenney, and R. Freter.** 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove

box procedure. Appl. Microbiol. 17:568–576.
2. **Bowden, G. H., J. M. Hardie, A. S. McKee, P. D. Marsh, F. D. Fillery, and G. L. Slack.** 1976. The microflora associated with developing carious lesions of the distal surfaces of the upper first premolars in 13–14 year old children, p. 223–242. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. 1. Information Retrieval, Inc., Washington, D.C.
3. **Bowden, G. H., J. M. Hardie, and G. L. Slack.** 1975. Microbial variation in approximal dental plaque. Caries Res. 9:253.
4. **Brown, L. R., S. Dreizen, S. Handler, and D. A. Johnston.** 1975. Effect of radiation-induced xerostomia on human oral microflora. J. Dent. Res. 54:740–750.
5. **Chapman, G. H.** 1946. The isolation and testing of faecal streptococci. Am. J. Digest. Dis. 13:105.
6. **Dennis, D. A., T. H. Gawronski, S. Z. Sudo, R. S. Harris, and L. E. A. Folke.** 1975. Variations in microbial and biochemical components of four-day plaque during a four-week controlled diet period. J. Dent. Res. 54:716–722.
7. **DeStoppelaar, J. D., J. van Houte, and O. Backer-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.
8. **Duaney, L. F., D. D. Zinner, and J. M. Jablon.** 1972. Epidemiologic studies of caries-active students. II. Diet, dental plaque and oral hygiene. J. Dent. Res. 51:727–733.
9. **Duchin, S., and J. van Houte.** 1978. Relationship of *Streptococcus mutans* and lactobacilli to incipient smooth surface dental caries in man. Arch. Oral Biol. 23:779–786.
10. **Ellen, R. P., and I. B. Balcerzak-Raczkowski.** 1975. Differential medium for detecting dental plaque bacteria resembling *Actinomyces viscosus* and *Actinomyces naeslundii*. J. Clin. Microbiol. 2:305–310.
11. **Feagin, F., T. Koulourides, and W. Pigman.** 1969. The characterization of enamel surface demineralization, remineralization, and associated hardness changes in human and bovine material. Arch. Oral Biol. 14:1407–1417.
12. **Folke, L. E. A., T. H. Gawronski, R. H. Staat, and R. S. Harris.** 1972. Effect of dietary sucrose on quantity and quality of plaque. Scand. J. Dent. Res. 80:529–533.
13. **Georg, L. K., A. Libero, and B. S. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab. Clin. Med. 44:422–428.
14. **Gold, O. C., H. V. Jordan, and J. van Houte.** 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. 18:1357–1365.
15. **Goldsworthy, N. E., and H. C. Spies.** 1950. The *Lactobacillus* count and its relation to dental caries. Aust. Dent. J. 3:318–330.
16. **Hemmens, E. S., J. R. Blayney, and R. W. Harrison.** 1941. The microbic flora of bacterial plaques removed from carious and noncarious dental enamel. J. Dent. Res. 20:29–38.
17. **Ikeda, T., H. J. Sandham, and E. L. Bradley.** 1973. Changes in *Streptococcus mutans* and lactobacilli in relation to the initiation of dental caries in Negro children. Arch. Oral Biol. 18:555–566.
18. **Keene, H. J., and I. L. Shklair.** 1974. Relationship of *Streptococcus mutans* carrier status to the development of carious lesions in initially caries free recruits. J. Dent. Res. 53:1295.
19. **Koulourides, T., and J. F. Volker.** 1964. Changes of enamel microhardness in the human mouth. Ala. J. Med. Sci. 1:435–437.
20. **Loesche, W. J., R. N. Hockett, and S. A. Syed.** 1972. The predominant cultivable flora of tooth surface plaque removed from institutionalized subjects. Arch. Oral Biol. 17:1311–1326.
21. **Loesche, W. J., J. Rowan, L. H. Straffon, and P. J. Loos.** 1975. Association of *Streptococcus mutans* with human dental decay. Infect. Immun. 11:1252–1260.
22. **Loesche, W. J., and L. H. Straffon.** 1979. Longitudinal investigation of the role of *Streptococcus mutans* in human fissure decay. Infect. Immun. 26:498–507.
23. **Loesche, W. J., and S. A. Syed.** 1978. Bacteriology of human experimental gingivitis: effect of plaque and gingivitis score. Infect. Immun. 21:830–839.
24. **Mikx, F. H. M., J. S. van der Hoeven, K. G. König, A. J. M. Plasschaert, and B. Guggenheim.** 1972. Establishment of defined microbial ecosystems in germ free rats. I. Effect of interaction of *Streptococcus mutans* or *Streptococcus sanguis* with *Veillonella alcalescens* on plaque formation and caries activity. Caries Res. 6:211–221.
25. **Minah, G. E., and W. J. Loesche.** 1976. Development of methods to analyze sucrose metabolism by small dental plaque suspensions, p. 491–520. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. 2. Information Retrieval, Inc., Washington, D.C.
26. **Mihah, G. E., and W. J. Loesche.** 1977. Sucrose metabolism in resting-cell suspensions of caries-associated and non-caries-associated dental plaque. Infect. Immun. 17: 43–54.
27. **Minah, G. E., and W. J. Loesche.** 1977. Sucrose metabolism by prominent members of the flora isolated from cariogenic and non-cariogenic dental plaques. Infect. Immun. 17:55–61.
28. **Ostrom, C. A., and T. Koulourides.** 1976. The intraoral cariogenicity test in young subjects. Caries Res. 10:442–452.
29. **Ostrom, C. A., T. Koulourides, F. Hickman, and J. R. McGhee.** 1977. Microbial characterization of an experimental cariogenic plaque in man. J. Dent. Res. 56:550–558.
30. **Purdell-Lewis, D., A. Groeneveld, and J. Arends.** 1976. Hardness tests on sound enamel and artificially demineralized white spot lesions. Caries Res. 10:201–215.
31. **Ritz, H. L.** 1967. Microbial population shifts in developing human dental plaque. Arch. Oral Biol. 12:1561–1568.
32. **Rogosa, M.** 1964. The genus *Veillonella*. I. General cultural, ecological, and biochemical considerations. J. Bacteriol. 87:162–170.
33. **Rogosa, M., R. F. Fitzgerald, M. E. MacKintosh, and J. A. Beeman.** 1958. Improved medium for selective isolation of *Veillonella*. J. Bacteriol. 76:455–456.
34. **Rogosa, M., M. I. Kirchevsky, and F. S. Bishop.** 1965. Truncated glycolytic system in *Veillonella*. J. Bacteriol. 90:164–171.
35. **Rogosa, M., J. A. Mitchell, and R. F. Wiserman.** 1957. A selective medium for the isolation and enumeration of oral lactobacilli. J. Dent. Res. 30:682–689.
36. **Shklair, K. L., H. J. Keene, and P. Cullen.** 1974. The distribution of *Streptococcus mutans* on the teeth of two groups of naval recruits. Arch. Oral Biol. 19:199–202.
37. **Shklair, I. L., H. J. Keene, and L. G. Simonson.** 1972. Distribution and frequency of *Streptococcus mutans* in caries active individuals. J. Dent. Res. 51:882.
38. **Snyder, M. L.** 1965. The bacteriology of caries resistance, p. 242–280. *In* G. E. W. Wolstenholme, and M. O'Connor (ed), Caries resistant teeth, Ciba Foundation Symposium. Little Brown & Co., Boston.
39. **Steven, R. H., and I. D. Mandel.** 1977. *Streptococcus mutans* serotypes in caries-resistant and caries-susceptible adults. J. Dent. Res. 56:1044.

40. **Svanberg, M., and W. J. Loesche.** 1977. The salivary concentration of *Streptococcus mutans* and *Streptococcus sanguis* and their colonization of artificial tooth fissures in man. Arch. Oral Biol. **22**:441–447.
41. **Theilade, E., O. Fejerskov, T. Karring, and J. Theilade.** 1978. A microbiological study of old plaque in occlusal fissures of human teeth. Caries Res. **12**:313–319.
42. **Tehilade, E., O. Fejerskov, W. Prackyabrued, and M. Kilian.** 1974. Microbiologic study on developing plaque in human fissures. Scand. J. Dent. Res. **82**:420–427.
43. **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.
44. **van Houte, J.** 1980. Bacterial specificity in the etiology of dental caries. Int. Dent. J. **30**:305–326.
45. **Wolstenholme, G. E. W., and M. O'Connor.** 1965. Caries resistant teeth, Ciba Foundation Symposium. Little Brown & Co., Boston.
46. **Wrobel, W. L., F. A. Catalanotto, and R. G. Walter.** 1978. Taste thresholds in caries-free and caries-active naval recruits. Arch. Oral Biol. **23**:881–885.
47. **Yengo, A. N., and I. D. Mandel.** 1972. Sucrose tasting and dental caries in man. Arch. Oral Biol. **17**:605–607.