INFECTION AND IMMUNITY, July 1977, p. 55-61

Vol. 17, No. 1
Printed in U.S.A.

Sucrose Metabolism by Prominent Members of the Flora Isolated from Cariogenic and Non-Cariogenic Dental Plaques

G. E. MINAH[1]* AND W. J. LOESCHE

School of Dentistry and School of Medicine, University of Michigan, Ann Arbor, Michigan 48109

Received for publication 9 February 1977

Sucrose metabolism by resting-cell suspensions of pure cultures of representative members of the predominant cultivable flora isolated from cariogenic and non-cariogenic dental plaque was investigated by means of radiochemical techniques. *Streptococcus mutans* utilized sucrose at a considerably faster rate than *S. sanguis*, *S. mitis*, *Actinomyces viscosus*, *A. naeslundii*, or *Lactobacillus casei*, forming lactic acid, intracellular polysaccharide, insoluble extracellular glucan, and lactic acid from intracellular polysaccharide catabolism at faster rates than the other bacteria. The *Actinomyces* formed more volatile acids than the streptococci, mostly acetic, and *S. sanguis* formed more soluble extracellular polysaccharide than the other bacteria. The metabolic activity of *S. mutans* resembled the pattern of sucrose metabolism of cariogenic plaque, whereas the metabolic activity of the *Actinomyces* species, the predominant members of non-cariogenic plaque flora, resembled the sucrose metabolism of non-cariogenic plaques.

In human dental caries quantitative, but not necessarily qualitative, differences exist between the microbiota of plaque covering an early carious lesion and plaque on a non-carious site of the same tooth (14, 22) or adjacent teeth (19, 24). Cariogenic plaques (CP) harbor high levels of *Streptococcus mutans*, low levels of *S. sanguis* (19), and moderate to high levels of *Actinomyces* species (22). Lactobacilli increase as the lesion progresses (14, 20). Non-cariogenic plaques (NCP) are colonized by low or negligible levels of *S. mutans*, moderate levels of *S. sanguis* (19), negligible *Lactobacillus* populations, and high levels of actinomyces (22). Abundant evidence from animal studies has identified *S. mutans* and dietary sucrose as cariogenic agents (4, 5, 11, 12, 16, 17). When sucrose metabolism of CP and NCP removed from the same tooth was compared, the CP utilized sucrose at a substantially higher rate than the NCP, forming more lactic acid, total volatile acids, and cell-bound and insoluble products per colony-forming unit, per milligram of plaque protein, or per particle count of plaque (22). This rapid metabolism of sucrose can in part explain the cariogenicity of the CP. The intention of the present investigation was to compare the in vitro sucrose metabolism of a pure culture of *S. mutans* with pure cultures of other numerically prominent bacterial species found in CP and NCP. From these data it should be possible to determine which, if any, of the pure cultures metabolizes sucrose in a manner analogous to that observed in the CP and NCP.

[1] Present address: Department of Microbiology, Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore, Baltimore, MD 21201.

MATERIALS AND METHODS

S. mutans (strain 10449, serotype *c*), *S. sanguis* (strain S-1), and recent isolates of *S. mitis*, *Actinomyces viscosus*, *A. naeslundii*, and *Lactobacillus casei* were studied. The latter three strains were isolated from plaque and identified as part of another investigation (20). *S. mitis* was isolated from dental plaque and identified by colony morphology, Gram stain, catalase activity, and failure to grow on mitis salivarius bacitracin agar (9). An individual colony of each bacterial type was transferred from MM10 sucrose blood agar (18) to 5 ml of Trypticase broth (Difco) containing 0.1% glucose and 0.01% sucrose. The cultures were grown anaerobically for 24 h at 37°C, harvested by centrifugation, washed two times in RTF (18), suspended in 10 ml of RTF, and dispersed by sonic treatment for 10 s under a nitrogen gas flow. A 0.2-ml amount of the bacterial suspension, containing approximately 5×10^7 cells, was incubated with 0.2 ml of 2 μCi of either [^{14}C]sucrose (glucose, uniformly labeled) or [^{14}C]sucrose (fructose, uniformly labeled) (New England Nuclear Corp.). Enough unlabeled sucrose was added to give a final concentration of 0.1% sucrose. These resting-cell suspensions were incubated in a vinyl anaerobic chamber (1) under an atmosphere of 85% N_2, 10% H_2, and 5% CO_2 for 45 min. The metabolism of both moieties of sucrose was assessed periodically by previously described techniques (21, 22). Accordingly, glucosyl- and fructosyl-derived lactic acid, soluble

extracellular polysaccharide (ECP), total cell-bound and insoluble products, intracellular polysaccharide (ICP), carbon dioxide, total volatile acids, acetic acid, propionic acid, and butyric acid were quantitated. Levels of insoluble extracellular glucan (ECG) and lactic acid from catabolized glucosyl- and fructosyl-derived ICP were also determined. All metabolic data were normalized according to colony-forming units on MM10 sucrose blood agar.

RESULTS

Lactic acid production. *S. mutans* formed considerably more lactic acid at all time intervals than the other bacteria (Fig. 1). At 45 min, *S. mutans* formed 13.5 nmol of sucrose equivalents of lactic acid as compared to 8.2 nmol produced by *A. viscosus*, the next highest acid former. All of the tested bacteria formed slightly more lactic acid from the fructosyl moiety of sucrose than from the glucosyl moiety.

Soluble ECP production. Soluble glucan and fructan synthesized by the pure cultures was quantitated after 15 and 45 min of incubation (Fig. 2). *S. sanguis* formed more soluble ECP than the other bacteria. The streptococci formed more soluble ECP than either the actinomyces or *L. casei*.

ECG formation. *S. mutans* formed two to three times more ECG than the other bacteria after 15 and 45 min of incubation (Fig. 3). *S. sanguis* and *L. casei* were the least active ECG producers. After the bacteria were incubated for an additional 6 h, the ECG levels decreased slightly for all organisms except *S. sanguis*.

Lactic acid from ICP catabolism. *S. mutans* formed twice as much lactic acid from ICP catabolism as either *A. viscosus* or *S. mitis*, the next highest producers (Fig. 4). Slightly more acid was derived from the fructosyl moiety of sucrose by the streptococci, whereas the actinomyces and *L. casei* produced acid derived almost equally from both hexose moieties of sucrose.

ICP formation. *S. mutans* formed substantially more ICP than *A. viscosus*, the next highest ICP former, whereas *S. sanguis* and *L. casei* produced the least amount of ICP. After an additional 6 h of incubation, there was about a 40 to 60% decrease in the labeled ICP by all cultures (Fig. 5).

Volatile acid production. The *Actinomyces* and *Lactobacillus* strains produced twice as much volatile acids as the three streptococcal species. *S. mutans* was the least active strain tested. Acetic acid was the major volatile acid produced by each species. The *Actinomyces* species formed over twice as much acetic acid as each *Streptococcus* species. *A. viscosus* and *A. naeslundii* produced two to three times as much propionic acid as each of the other bacteria. *L. casei* produced the most butyric acid, whereas the streptococci did not produce detectable butyric acid (Fig. 6).

Distribution of radioactivity. The distribution of by-products of sucrose metabolism by the pure cultures, calculated as the percentage of the sucrose consumed at 45 min, is shown in Table 1. *S. mutans, A. viscosus, and S. mitis,* in that order, consumed more sucrose at each time period than the other strains. Half of the sucrose consumed by *S. mutans* went into acids primarily lactic, whereas the majority of the remainder appears as polysaccharides. *S. mutans* formed more lactic acid, ICP, and ECG on

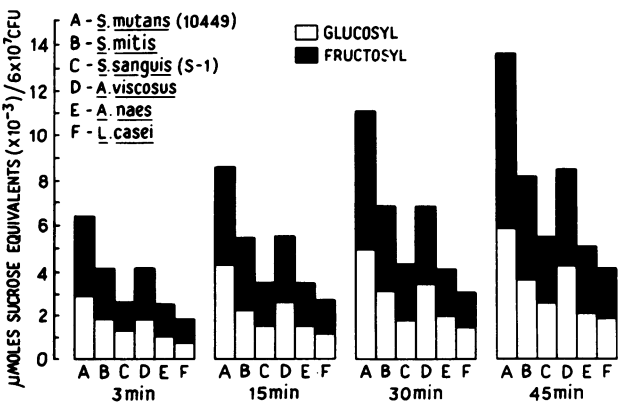

FIG. 1. *Lactic acid production by dental plaque bacteria during 45 min of incubation. Lactic acid was isolated from incubation mixtures by thin-layer chromatography (cellulose; 88% formic acid-2-butanone-3-butanol-water, 15:30:40:15). Lactic acid spots were removed from the chromatograms and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

both a relative and absolute basis than the other strains. *S. sanguis* was distinctive for its high proportion of soluble ECP. *A. viscosus, A. naeslundii,* and *L. casei* produced proportionately higher levels of volatile acids.

This pattern of metabolism was discernible at the earlier time periods, as illustrated in Fig. 7, where the proportional contributions of each end product at 3, 15, 30, and 45 min are displayed.

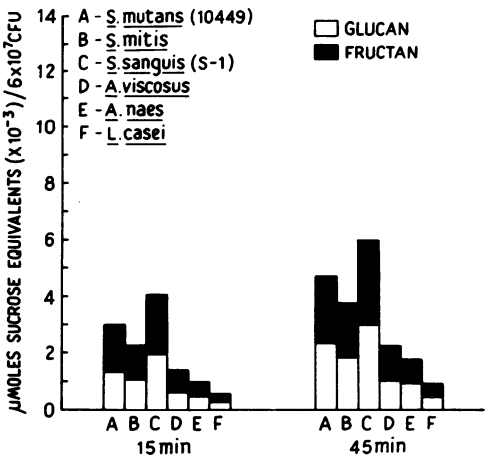

FIG. 2. *Soluble ECP production by dental plaque bacteria. Portions of resting-cell suspensions were filtered (Nuclepore filters, 0.2 μm). The filtrates were dried on glass fiber disks. The disks were washed for 90 min in absolute methanol (methanol was changed twice), dried, and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

DISCUSSION

The objective of this investigation was to determine the sucrose metabolism of resting-cell suspensions of pure cultures of bacteria that are representative of the dominant cultivable flora in CP and NCP. In this manner, we sought to

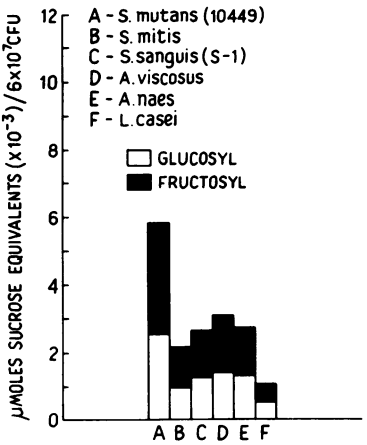

FIG. 4. *Lactic acid released from dental plaque bacteria during ICP catabolism. Portions of resting-cell suspensions of dental plaque were filtered (Nuclepore filters, 0.2 μm). Filters with retentates were incubated for 6 h at 37°C in RTF containing 2% sucrose and 2% sodium lactate. Lactic acid from ICP catabolism was isolated from samples of the incubation mixture by thin-layer chromatography (cellulose; 88% formic acid-2-butanone-3-butanol-water, 15:30:40:15) and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

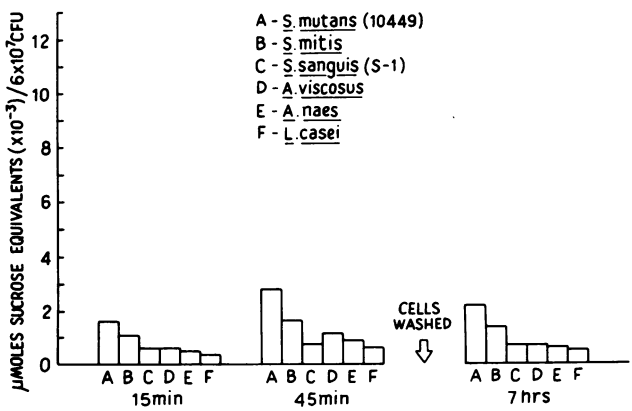

FIG. 3. *ECG formation by dental plaque bacteria. Portions of resting-cell suspensions were filtered (Nuclepore filters, 0.2 μm) at the times indicated. Filters with retentates were exposed to Dowex 50W in 0.2 N HCl for 48 h at 100°C. Glucose in the hydrolysates was isolated by thin-layer chromatography (cellulose; 88% formic acid-2-butanone-3-butanol-water, 15:30:40:15) and counted by liquid scintillation. ECG was calculated by the following formula: ECG = total glucose − ICP. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

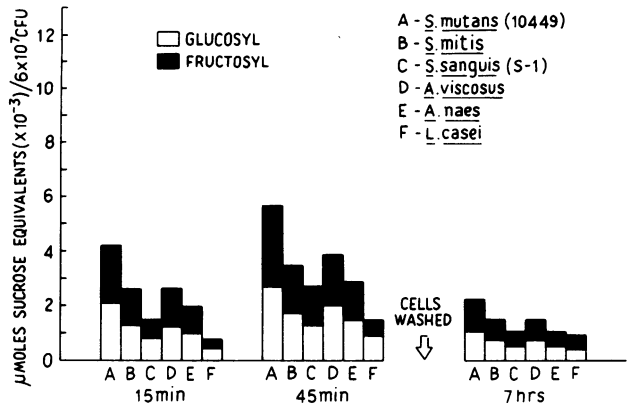

FIG. 5. *ICP formation by dental plaque bacteria during 45 min of incubation. Portions of resting-cell suspensions were filtered at 15 and 45 min (Nuclepore filters, 0.2 μm). Filters with retentates were exposed to Dowex 50W in 0.2 N HCl for 48 h at 100°C. Glucose in the hydrolysates was isolated by thin-layer chromatography (TLC) (cellulose; 88% formic acid-2-butanone-3-butanol-water, 15:30:40:15). Other portions of the resting-cell suspensions were filtered (Nuclepore filters, 0.2 μm) at 45 min of incubation. The filters with retentates were incubated for 6 h at 37°C in RTF containing 2% sucrose and 2% sodium lactate prior to hydrolysis and the TLC isolation of glucose. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

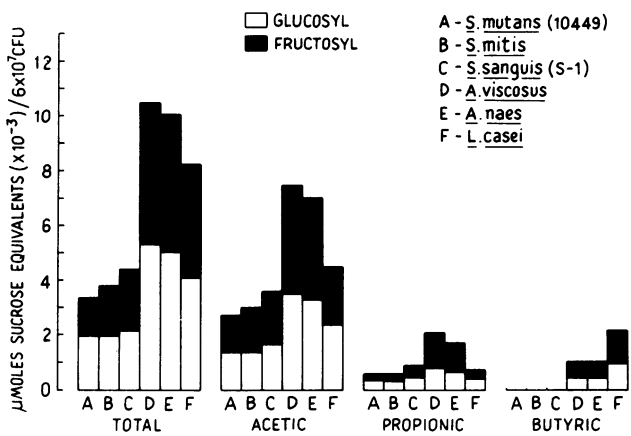

FIG. 6. *Volatile acid formation by dental plaque bacteria during 45 min of incubation. Portions of resting-cell suspensions were incubated in the outer wells of Conway dishes for 45 min. The sample was combined with 1.0 N HCl in the outer wells for 15 min to liberate CO_2. The acidified sample was then combined with sodium sulfate in the outer wells, and the dishes were incubated for 48 h at 37°C. KOH (5.0%) in the center wells of the Conway dishes, containing salts of the diffused volatile acids, was counted by liquid scintillation. Other portions of the resting-cell suspensions were combined with 5% KOH (20 μl) and dried. A mixture of concentrated acetic, propionic, and butyric acids in concentrated HCl (50 μl, total volume) was added. Five microliters of this mixture was injected into a Varian Aerograph (model 2700) gas-liquid chromatographic unit. As peaks of the carrier acids appeared on the recorder, 90% of the effluent was collected via a fraction splitter in Pasteur pipettes containing glass wool moistened with 10% sodium carbonate. The glass wool was removed and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products.*

determine if any of the individual bacterial types could duplicate the pattern of sucrose metabolism observed in resting-cell suspensions of CP and NCP (22). Our prior investigation indicated that in CP the hierarchy of cultivable bacteria was *S. mutans* > *Actinomyces* sp. > *S. mitis* > gram-positive rods > *Veillonella* sp. > *S. sanguis*, and in NCP the hierarchy was *Actinomyces* sp. ≥ *Veillonella* sp. > *S. sanguis* > *S. mitis* > *S. mutans*. From this array we selected strains of *S. mutans* (serotype *c*) and *S. mitis* as representative of CP and *A. viscosus*, *A. naeslundii*, and *S. sanguis* as representative of NCP. *L. casei* was tested because of

its association with dental caries (20). *Veillonella* sp. were prominent in both plaque types, but because they do not ferment sucrose they were not included in these experiments.

S. mutans was the most saccharolytic of the six strains tested, forming more lactic acid, ICP, lactic acid from ICP, and ECG than any of the other bacteria (Table 2). This is in agreement with another investigation (23), which found *S. mutans* to decrease the pH more rapidly and to form more lactic acid than other oral streptococci. The values for volatile acids, water-soluble ECP, ECG, and the glucose-to-fructose ratio in lactic acid compare favorably with those found by Tanzer et al. (26) using column chromatography and KOH extractions to isolate the end products. Tanzer et al. found lactic acid to account for about 80% of the consumed sucrose, whereas in the present investigation it accounted for about 40%. ICP was not quantitated by these investigations, and it is possible that under the conditions of their incubation the ICP was converted to lactic acid or not formed at all. Our investigation was performed with serotype *c* strain, whereas Tanzer et al. used a serotype *d* strain of *S. mutans*, so that the differences observed may reflect the genetic differences between these serotypes (3).

A. viscosus and *A. naeslundii* ranked second and fourth in regard to the amount of sucrose consumed (Table 2). These organisms did not form as much lactic acid, ICP, lactic acid from ICP, and ECG as did *S. mutans*. Their main end products were volatile acids, especially acetic acid.

S. mitis was the third most active consumer

TABLE 1. *End products of sucrose metabolism by resting-cell suspensions of various plaque bacteria after 45 min of incubation*

Organism	Amt of sucrose consumed (μmol × 10^{-3}/6 × 10^7 CFU)[a]	% of sucrose consumed that is recovered in:											
		Acids				Polysaccharides					CO_2	Free glucose	Total accounted for
		Lactic	Volatile	Acetic	Total lactic + volatile	Soluble ECP	Cell-bound ECP	ICP	ECG	Total ECP			
S. mutans	36	40	8	6	48	13	30	20	9	43	3	3	97
S. mitis	26	33	15	10	48	17	24	14	7	41	3	2	94
S. sanguis	21	23	20	15	43	25	23	13	6	48	5	2	98
A. viscosus	28	30	35	27	65	11	20	11	6	31	5	2	103
A. naeslundii	22	25	40	30	65	9	20	11	6	29	5	2	101
L. casei	17	22	35	20	57	11	25	12	5	36	3	1	97

[a] CFU, Colony-forming units.

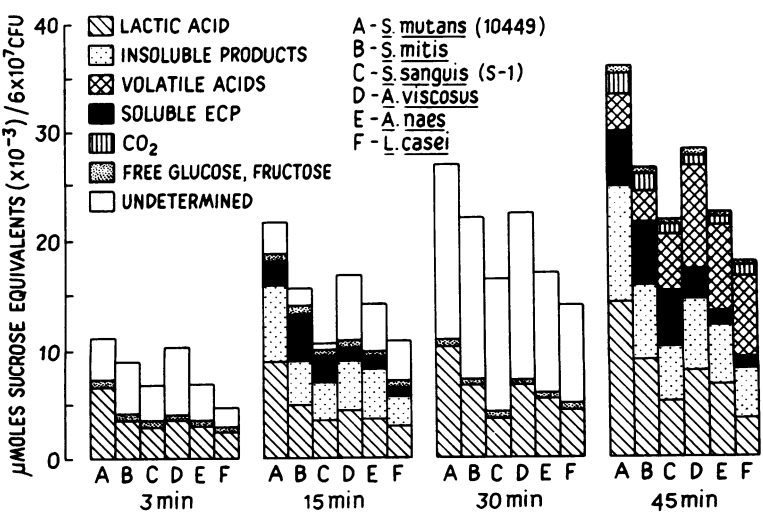

FIG. 7. *Distribution of by-products of sucrose metabolism by dental plaque bacteria. The height of each bar represents the amount of sucrose consumed during the particular time interval. The height of each bar segment represents the proportion of the consumed sucrose recovered as the products indicated by the code on the figure.*

TABLE 2. *Comparison of sucrose metabolism by CP and NCP and by pure cultures isolated from plaque*

Sucrose metabolism by:	μmol sucrose equivalents × 10^{-3}/6 × 10^{7} CFU[a]					
	Sucrose consumed	Lactic acid	Volatile acids	Soluble ECP	ICP	Cell-bound ECG
CP[b]	72	26	17	7	12	4
NCP[b]	48	14	14	5	9	2
S. mutans	36	14	3	5	7	3
S. mitis	26	9	4	4	4	2
S. sanguis	21	5	4	5	3	1
A. viscosus	28	8	10	3	3	2
A. naeslundii	22	6	9	2	2	1
L. casei	17	4	6	2	2	1

[a] CFU, Colony-forming units.
[b] Values taken from reference 22.

of sucrose. It formed proportionately more volatile acids and soluble ECP than did *S. mutans* and proportionately less lactic acid and ICP (Table 1). *S. sanguis* and *L. casei* were the least active metabolizers of sucrose. Of particular interest was the relatively high proportion of sucrose that was converted into soluble ECP by *S. sanguis* (Table 1). As soluble ECP are not considered to contribute to the caries process (7), this finding may in part explain the nonassociation of *S. sanguis* with human caries (19).

These data suggest that *S. mutans* was responsible for most of the differences in sucrose metabolism between CP and NCP. These findings should, however, be interpreted with caution for several reasons. First, pure cultures lack the metabolic diversity and interactions of a mixed culture. Second, the bacterial strains studied were not necessarily identical to strains of the same species found in plaque. Third, the enzyme profile of the laboratory strains grown in enriched media may differ qualitatively and quantitatively from the enzyme profile found in the same bacteria in vivo. Fourth, the laboratory strains were harvested from early-stationary-phase cultures, whereas in plaque all growth phases could be present.

The pure cultures were sluggish in their utilization of sucrose compared to the CP and NCP (Table 2). A similar phenomenon was noted previously when cells of *S. mitis* grown in vivo metabolized glucose two to three times faster than cells grown in vitro (10). Apparently, in vivo the cells are primed for rapid metabolism of a substrate when it becomes available. This raises another cautionary note in terms of comparing sucrose metabolism by the pure cultures with sucrose metabolism by plaque suspensions.

Yet despite these transposition problems, the pure culture results show *S. mutans* in its metabolism of sucrose to most resemble sucrose metabolism by the CP, and the *Actinomyces* sp. and *S. sanguis* in their metabolism of sucrose resemble sucrose metabolism by the NCP (Table 2). High rates of sucrose utilization, lactic acid formation, ICP, and cell-bound ECG formation are distinguishing characteristics of CP and *S. mutans*. A lower rate of sucrose utilization, accompanied by proportionately higher levels of volatile acids and soluble ECP formation, distinguishes the NCP, *Actinomyces* sp., and *S. sanguis*. These data contribute to the argument that *S. mutans* is an important dental pathogen in humans but do not preclude the involvement of other plaque organisms in the decay process.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants DE 03423 and DE 03011 from the National Institute of Dental Research. G.E.M. was a recipient of Public Health Service fellowship award DE 00013 from the National Institute of Dental Research.

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