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Sucrose Metabolism in Resting-Cell Suspensions of Caries-Associated and Non-Caries-Associated Dental Plaque

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Small specimens of cariogenic plaque (CP) and non-cariogenic plaque (NCP) from the same tooth were individually dispersed in buffer, divided equally, and incubated for 45 min with [^{14}C]sucrose uniformly labeled either in the glucosyl moiety or the fructosyl moiety. Sucrose metabolism was analyzed periodically during an anaerobic incubation at 37°C. Radiochemical techniques were devised to analyze formation of lactic acid, soluble extracellular polysaccharide, total cell-bound and insoluble products, intracellular polysaccharide, lactic acid from intracellular polysaccharide catabolism, insoluble extracellular glucan, CO_2, total volatile acids, individual volatile acids, and rates of sucrose consumption. The contribution of the glucosyl and fructosyl moieties of sucrose to each metabolic by-product was determined. All of the metabolic data were adjusted to the size of the plaque specimens as determined by colony-forming units, Coulter counter particle counts, and fluorometric protein analyses. Both types of dental plaque transformed from 70 to 80% of the consumed sucrose into lactic acid and cell-bound and insoluble products, primarily intracellular polysaccharide and extracellular glucan. Volatile acids accounted for most of the remaining by-products. CP metabolized significantly more sucrose than NCP and consequently produced significantly higher levels of each metabolic by-product. High levels of *Streptococcus mutans* were found in CP (averaging 40% of colony-forming units), whereas it was virtually absent in NCP. *Actinomyces* and *S. sanguis* levels were distinctly higher in NCP. NCP harbored more anaerobes and dextranase-forming microorganisms than CP.

Streptococcus mutans is a dental pathogen in the rodent model (6, 7, 13, 30-32). In humans, *S. mutans* is usually present in plaque associated with early carious lesions and is found in low or negligible levels in plaque covering noncarious surfaces (5, 27-29, 32, 33, 37, 44, 45, 54). Organic acids, primarily lactic, and a variety of polysaccharides are formed by *S. mutans* in sucrose broth cultures (2, 12, 13, 15-18, 50-52). Some of these metabolic by-products have been implicated as agents responsible for *S. mutans* virulence (12, 16, 18, 36, 51) but have not been isolated and quantitated in vivo in plaques containing elevated proportions of *S. mutans*. Techniques for studying sucrose metabolism of small dental plaque specimens have been developed (40) and were used in this investigation to compare sucrose metabolism in plaque removed at the same time from a carious lesion and a noncarious site on the same tooth.

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MATERIALS AND METHODS

Collection of dental plaque. Small dental plaque specimens were removed with sterile periodontal scalers from teeth present in 12 caries-active children, 5 to 7 years old, at the pedodontic clinic of the University of Michigan School of Dentistry. Each child exhibited at least 12 untreated carious surfaces as determined visually and radiographically. The specimen designated as cariogenic plaque (CP) was removed from an approximal lesion of a primary molar. The specimen designated as non-cariogenic plaque (NCP) was removed from a nearby noncarious surface on the same tooth. Each specimen was dispersed for 10 s by ultrasound (Sonifier cell disruptor, model 21850) under nitrogen in 0.4 ml of reduced transport fluid (RTF) (35) and introduced into a vinyl anaerobic glove box containing 85% N_2, 10% H_2, and 5% CO_1 (1).

Incubation mixtures. The samples were divided equally and placed into 2-dram (ca. 2.4-g) vials. A 2-μCi amount of [^{14}C]sucrose (uniformly labeled in the glucosyl moiety; New England Nuclear Corp.) in 0.2 ml was added to one-half of each sample, and 2 μCi of [^{14}C]sucrose (uniformly labeled in the fructosyl moiety; New England Nuclear Corp.) in 0.2 ml was added to the other half. The final sucrose concentra-

tion of the incubation mixtures was made to 0.06%. Metabolic by-products were analyzed at 3, 15, 30, and 45 min during a 45-min incubation by techniques described in detail previously (40) and summarized below and in Table 1.

Quantitation of the specimens. Each dental plaque specimen was quantitated by total colony-forming units (CFU) on MM10 sucrose-blood agar (35) by total particle counts, using a Coulter counter (model ZBI), and by protein content, using the Fluram (Fluorescamine) reagent (53). The size of all specimens ranged from 10^7 to 5×10^7 CFU, 0.33×10^8 to 1.66×10^8 particles, and 25 to 125 g of protein.

Bacteriological procedures. Portions (50 μl) of the resting-cell suspensions were serially diluted in RTF and plated on MM10 sucrose-blood agar (for aerobic and anaerobic incubation), mitis salivarius bacitracin agar (a selective medium for *S. mutans* [20]), and a blue dextran agar (MM10 without blood or sucrose and with blue dextran as the only carbohydrate). In the latter medium dextranase-forming colonies formed light halos in the dark blue medium (46). The RTF and the solid media were prereduced in the anaerobic chamber for at least 24 h before use. After 10 to 14 days of incubation, the CFU on each medium were enumerated along with the counts of the recognizable colony types. Each colony on the anaerobically incubated MM10 sucrose-blood agar plates, which displayed from 50 to 100 colonies and did not resemble typical *S. mutans*, *S. sanguis*, or *S. salivarius* colonies, was tested for catalase activity with 10% hydrogen peroxide and was Gram stained.

The isolates were separated into the following groups: *S. mutans*, *S. sanguis*, and *S. salivarius*, i.e., gram-positive cocci in chains with a characteristic colony morphology on MM10 sucrose-blood agar; other streptococci species, i.e., gram-positive, catalase-negative cocci in chains without a characteristic colonial morphology on MM10 sucrose-blood agar; *Actinomyces viscosus*, i.e., a gram-positive pleomorphic rod that was catalase positive; *Veillonella* species, i.e., gram-negative, catalase-negative cocci; *Neisseria* species; gram-negative, catalase-positive cocci; gram-negative rods; gram-positive rods and yeast. Catalase-negative *Actinomyces* species were differentiated on the basis of colonial and microscopic morphology from the other gram-positive rods.

TABLE 1. *Sequence of procedures*

Procedure	μl	
Samples added to suspension		
1. Plaque collected and placed in 400 μl of RTF		
2. Plaque dispersed by sonic treatment		
3. Plaque equally divided	200	200
4. Radioactive sucrose added		
a. Sucrose with [^{14}C]glucose	200	
b. Sucrose with [^{14}C]fructose		200
5. Addition of unlabeled sucrose		
a. 5 μl of 5% sucrose	5	5
Starting volume containing 0.062% sucrose	405	405
Samples removed for various measurements		
6. Bacterial culturing	50	50
7. Protein measurement	20	20
8. Particle count in Coulter counter	50	50
9. Lactic acid and sugars (TLC)[a] (4 determinations)	4	4
10. Soluble polysaccharide and cell-bound products (2 determinations)	100	100
11. Lactic acid from ICP	50	50
12. Carbon dioxide	50	50
13. Volatile acids	50	50
Total volume removed	374	374

[a] TLC, Thin-layer chromatography.

Soluble products. The rates of sucrose consumption, free glucose and free fructose production, and lactic acid formation were analyzed by thin-layer chromatography. One-microliter portions of the incubation mixtures were spotted on vinyl-backed cellulose thin-layer chromatography sheets (Eastman) at 3, 15, 30, and 45 min. Cold carrier compounds (1 μl of a mixture of 2% sucrose, 2% glucose, 2% fructose, and 2% sodium lactate) were spotted at the origin. The chromatograms were developed with 88% formic acid–2-butanone–3-butanol–water (15:30:40:15) (47). The sugar spots were visualized with aniline-diphenylamine spray, and the lactic acid spots were visualized with a bromocresol green, bromophenol blue-potassium permanganate spray (47). Spots were cut from the chromatograms with scissors, placed in scintillation fluid (P-dioxane, 2,5-diphenyloxazole, naphthalene), and counted in a Beckman LS-100C scintillation counter.

Soluble extracellular polysaccharides (ECP) were quantitated in filtrates of the incubation mixtures. Portions (50 μl) were filtered through 0.2-μm-pore size Nuclepore filters at 15 and 45 min. The filtrates were dried on glass fiber disks (Whatman GF/C), which were then washed in absolute methanol for 90 min (11) (the methanol was changed every 30 min). The labeled soluble ECP precipitated in the disks was measured by liquid scintillation counting of the disks (40).

Insoluble products. Insoluble material retained on the filters after the filtration step was hydrolyzed in Dowex 50W in 0.2 N HCl (40%, wt/vol, suspension of Dowex 50W in 0.2 N HCl) for 48 h at 100°C (40). The Dowex was removed by filtration through glass wool in Pasteur pipettes. Glucose and fructose in the hydrolysate were separated by the thin-layer chromatography procedure described above. It was assumed that most of the labeled glucose would be derived from intracellular polysaccharides (ICP) and cell-bound and/or insoluble extracellular glucan (ECG). Most of the labeled fructose would be derived from cell-bound extracellular fructan.

Intracellular polysaccharide. At 45 min, 50 μl of each incubation mixture was filtered through 0.2-μm-pore size Nuclepore filters. The retentates on the filters were incubated for 6 h at 37°C in RTF containing 2% sodium lactate and 2% sucrose. The unlabeled lactate and sucrose reduced the utilization of labeled lactic acid and ECP, respectively, by plaque bacteria (40). Hence, the labeled lactic acid

found in the incubation mixture would be derived from ICP. This lactic acid was isolated by thin-layer chromatography methods described above and quantitated.

The ICP and ECG levels were calculated from the ratio of fructosyl- and glucosyl-derived lactic acid produced during ICP catabolism and the level of fructosyl-derived glucose in the plaque hydrolysates (40).

Carbon dioxide. At 3 min, a portion (50 μl) of each resting-cell suspension was introduced into the outer well of a Conway dish (4), which contained 200 μl of 1.0 N HCl in the outer well and 500 μl of 1.0 N KOH in the inner well. After 45 min of incubation, the incubation mixture and the acid were combined. After an additional 15 min, the contents of the center well were removed and counted in the scintillation counter. The level of radioactivity served as a measure of carbon dioxide production.

Total volatile acid. After the KOH containing labeled carbon dioxide was removed from the Conway dish, fresh KOH was introduced into the center well and the cover of the Conway dish was repositioned. The acidic sample was then combined with 0.5 g of sodium sulfate that had been placed in the outer well (4). The volatile acids, which were allowed to diffuse into the KOH in the center well over a period of 48 h, were quantitated by scintillation counting of the center well contents and washings.

Individual volatile acids. After 45 min of incubation, portions (50 μl) of the resting-cell suspensions were placed in 0.5-dram (ca. 0.6-g) vials containing 20 μl of the 1.0 N KOH. The contents of these vials were subsequently dried in an oven at 100°C. Fifty microliters of a standard mixture of volatile acids (equal portions of 20% acetic, propionic, and butyric acids in concentrated HCl) was added to the vials containing the dried, labeled volatile acids. The standard volatile acids served to reacidify the unknown acids and as carrier compounds for the labeled acids in the plaque samples. A 5-μl portion was injected with a Hamilton syringe into a Varian Aerograph (model 2700) gas-liquid chromatography unit equipped with a flame ionization detector, a fraction splitter, which allowed collection of 90% of the effluent intact, and a 20 M carbowax plus 3% phosphoric acid column. Operating conditions were as follows: column temperature, 135°C; detector temperature, 20°C; injector temperature, 180°C; carrier gas flow, 25 ml/min. As the peaks of acetic, propionic, and butyric acids appeared on the recorder, the effluent was collected in 10% sodium carbonate-soaked glass wool in Pasteur pipettes. The glass wool, containing salts of the individual labeled acids, was counted by liquid scintillation. A computer (Varian CDS 101) permitted integral analysis of the peaks.

Calculations and statistics. The radioactive counts and other measurements were entered into a specially written computer program. The raw data were normalized on the basis of (i) anaerobic viable count, (ii) particle count, and (iii) protein content (in micrograms). These normalized data provided the values for the subsequent statistical analysis. The difference in the quantities of metabolic by-products formed in the CP and NCP from each subject was tested by the Wilcoxon signed rank test, a nonparametric statistical procedure. The use of this test permitted a comparison of the metabolic activity in dental plaque from two sites of an individual tooth. Variables such as dietary differences, salivary chemistry and defense mechanisms, time proximity of exposure of the plaque to a particular substrate, and the presence of metabolic inhibitors in the dental enamel (such as fluoride content) would not affect a test of significance by the Wilcoxon signed rank test.

RESULTS

Bacteriological findings. Approximately 99% of the cultivable plaque flora was identified at either the species or genus level (Table 2). In the CP, *S. mutans* averaged 40% and *S. sanguis* averaged 0.8% of the CFU. In the NCP, *S. mutans* averaged 0.5% and *S. sanguis* averaged 7.2% of CFU. The presence of *S. mutans* in CP and *S. sanguis* in NCP was a consistent finding. The total streptococci averaged 52% of the CP flora and 13.3% of the NCP flora. The levels of *Actinomyces* averaged 24% of CFU in the CP and 59% in the NCP. In the CP, *Veillonella* and gram-positive rods averaged 7 and 11% of CFU, respectively, and in the NCP, 17% and not detectable, respectively. The differences in the levels of *S. mutans*, *S. sanguis*, total streptococci, *Actinomyces* species, and gram-positive rods in CP and NCP were significant ($P = 0.05$, Wilcoxon signed rank rest; Table 2). The dextranase formers averaged 6.6% of the CFU in the NCP and 3.4% of the CFU in the CP (Table 2). This difference was significant ($P < 0.05$, Wilcoxon signed rank test; Table 2). The ratio of anaerobic CFU to aerobic CFU was significantly higher in NCP than in CP ($P < 0.05$, Wilcoxon signed rank test; Table 2). The mitis salivarius bacitracin agar verified the *S. mutans* levels on MM10 sucrose-blood agar.

Sucrose consumption. The CP utilized sucrose at a significantly higher rate than NCP at each time period (Fig. 1) ($P < 0.001$, Wilcoxon signed rank test) whether the data were normalized on the basis of 6×10^7 CFU, 2×10^8 particles, or 150 μg of protein. These standard values, 6×10^7 CFU, 2×10^8 particles, and 150 μg of protein, approximated actual CFU, particle number, and protein content of the plaque specimens, respectively. All incubation mixtures contained excess sucrose at 45 min. Considerable intersubject variation occurred, as evidenced by the fact that the standard deviations ranged from 24 to 31% of the mean of values for each time period.

Lactic acid production. In CP approximately 36% of the sucrose consumed at each

TABLE 2. *Predominant cultivable bacteria in CP and NCP*

Bacteria	CFU (%)[a]					
	CP (n = 17)			NCP (n = 17)		
	Avg ± SD	Range	Frequency	Avg ± SD	Range	Frequency
S. mutans	40.1 ± 22.3[b]	13.2-84.4	17/17	0.5 ± 1.7	0-6.8	3/17
S. sanguis	0.8 ± 1.5[b]	0-5.3	7/17	7.2 ± 8.2	0-27.6	14/17
S. mitis	11.2 ± 10.5	0-30.4	13/17	5.6 ± 7.0	0-28	13/17
A. viscosus	4.3 ± 13.1	0-52	3/17	8.7 ± 14.6	0-45.8	11/17
Actinomyces sp.	24.1 ± 23.1[b]	0-63	12/17	60 ± 21.7	21-92.5	17/17
Veillonella sp.	7.2 ± 7.6	0-21.2	10/17	16.2 ± 12.4	0-36.6	13/17
Gram-positive rods	11.1 ± 17.6[b]	0-52.5	9/17	0	0	0/17
Dextranase formers	3.4[b]	0.0-10.5		6.6	0.0-18.8	
Anaerobe/aerobe ratio	5.6[b]	1-18.2		9.2	1.9-32.2	

[a] The bacteria were identified by colonial morphology, Gram stain, and catalase activity and are reported as a percentage of the anaerobic viable count on MM10 sucrose-blood agar. SD, Standard deviation.
[b] The differences between CP and NCP are significant: $P < 0.05$, Wilcoxon signed rank test.

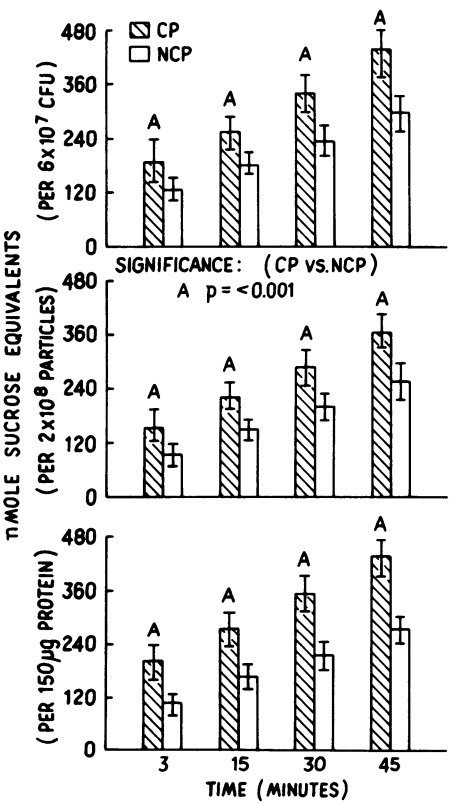

FIG. 1. *Sucrose consumption in CP and NCP. Sucrose was isolated from incubation mixtures of dental plaque by thin-layer chromatography. Sucrose spots were cut from the chromatograms and counted by liquid scintillation. The height of the bars gives the average and the brackets give the standard deviation. A = when the differences between CP and NCP were significant; $P < 0.001$, Wilcoxon signed rank test.*

time period was converted into lactic acid. In NCP approximately 30% of the sucrose consumed at each time period was converted into lactic acid. CP produced lactic acid at a significantly higher rate at each time period than NCP when the data were normalized to the three reference parameters ($P < 0.0001$, Wilcoxon signed rank test) (Fig. 2). About 42% of the lactic acid formed in CP and 36% in NCP were derived from the glucosyl moiety of sucrose. Fifty-eight and 64% of the lactic acid in CP and NCP, respectively, were derived from the fructosyl moiety of sucrose. The glucose (G)-to-fructose (F) ratio in lactic acid was 0.72 to 1 in CP and 0.56 to 1 in NCP. Standard deviations ranged from 26 to 36% of the means of values for each time period.

Free glucose and fructose production. In both plaque types, free glucose and fructose levels represented from 0.5 to 1.0% of the sucrose consumed at each time period. Free glucose levels were slightly but not significantly higher than free fructose levels in both plaque types.

Soluble ECP production. In both plaque types, approximately 10% of the sucrose at 15 and 45 min resulted in soluble ECP formation. CP formed significantly more soluble ECP than NCP at both time periods when plaque was quantitated by CFU and protein content ($P < 0.05$, Wilcoxon signed rank test; Fig. 3). More fructan than glucan was formed in both plaque types; i.e., the glucan-to-fructan ratio was 0.89 to 1 in CP and 0.79 to 1 in NCP. Standard deviations of values for each time period ranged from 27 to 33% of the mean of these values.

Total cell-bound and insoluble products. In both plaque types, approximately 43% of the

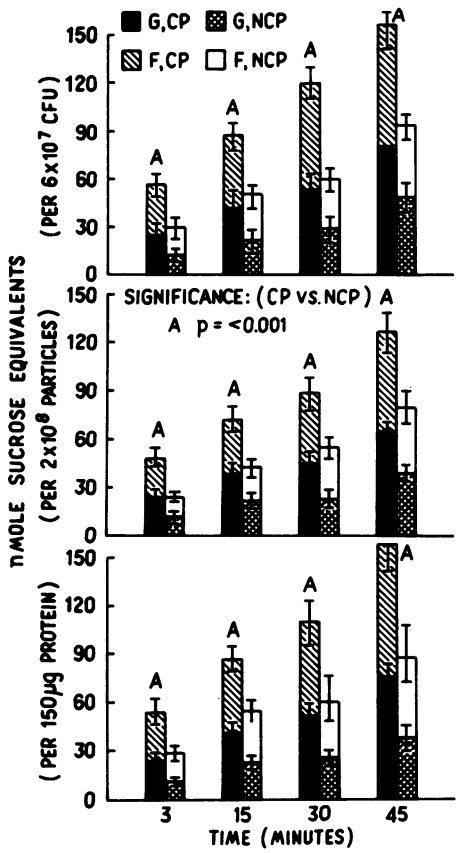

FIG. 2. *Lactic acid production in CP and NCP during 45 min of incubation. Lactic acid was isolated from incubation mixtures by thin-layer chromatography. Lactic acid spots were removed from chromatograms and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each sector gives the average value and the brackets give the standard deviation. G is lactate derived from the glucosyl moiety of sucrose and F is lactate derived from the fructosyl moiety of sucrose. A = when the differences between CP and NCP were significant, $P < 0.01$.*

sucrose consumed at 15 min, and 30% at 45 min, were transformed into cell-bound and insoluble products. CP formed significantly more cell-bound and insoluble products than NCP at 15 and 45 min ($P < 0.001$, Wilcoxon signed rank test; Fig. 4). In both plaque types slightly more than half of the total cell-bound and insoluble products was derived from the fructosyl moiety of sucrose. The G-to-F ratio in cell-bound and insoluble products was 0.96 to 1 in CP and 0.92 to 1 in NCP. Standard deviations of values at each time period ranged from 25 to 35% of the mean of values for each time period.

Lactic acid from ICP catabolism. The CP released significantly more lactic acid than the NCP ($P < 0.001$, Wilcoxon signed rank test) during the period of ICP catabolism (Fig. 5). Both plaque types elaborated more lactic acid derived from the fructosyl moiety of sucrose than that from the glucosyl moiety of sucrose. The G-to-F ratios in lactic acid from ICP were 0.92 in the CP and 0.89 in the NCP. Standard deviations of values for each time period ranged from 24 to 33% of the means of these values.

ICP formation. ICP was calculated from the values of the fructosyl-derived glucose in plaque hydrolysates obtained from cells given

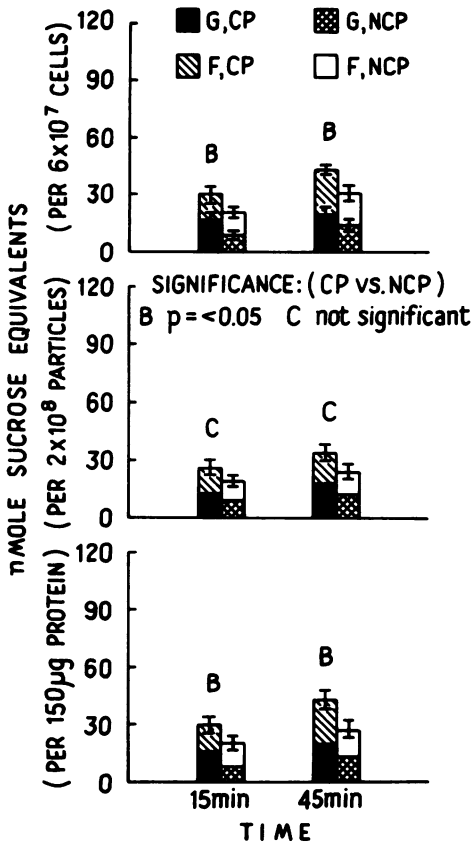

FIG. 3. *Soluble ECP formation in CP and NCP during 15 and 45 min of incubation. Portions of dental plaque resting-cell suspensions were filtered, and the filtrates were dried on glass fiber disks. The disks were washed for 90 min in absolute methanol, dried, and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each sector gives the average value and the brackets give the standard deviation. G = ECP derived from the glucose moiety of sucrose, F = ECP derived from the fructose moiety of sucrose, B = when the differences between CP and NCP were significant ($P < 0.05$), and C = no significant differences.*

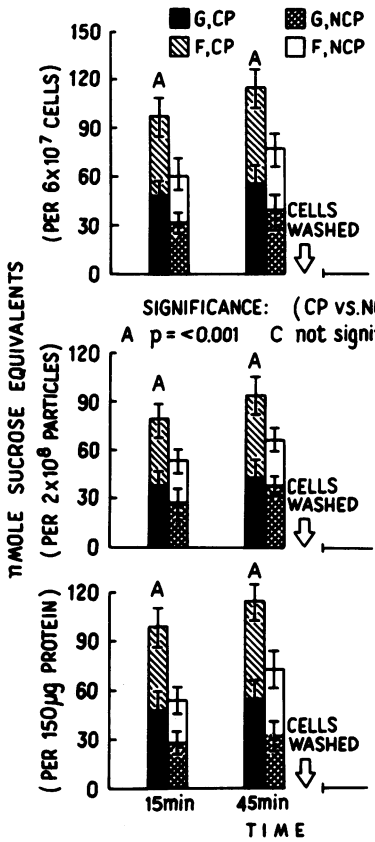

FIG. 4. *Total cell-bound and insoluble products formed in CP and NCP during 45 min of incubation. Portions of resting-cell suspensions of dental plaque were filtered. The filters with retentates were exposed to Dowex 50W in 0.2 N HCl for 48 h at 100°C. Radioactivity in the hydrolysates represented formation of cell-bound and insoluble products. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each segment gives the average value and the brackets give the standard deviation. G = polysaccharide derived from the glucose moiety of sucrose and F = polysaccharide derived from the fructose moiety of sucrose. A = when the differences between CP and NCP were significant, $P < 0.01$.*

sucrose labeled in the fructosyl moiety and the G-to-F ratio in lactic acid from ICP catabolism (40). In the CP, 72% of the total cell-bound and insoluble glucose at 15 min and 68% at 45 min was ICP. In NCP, ICP comprised 78% of the total cell-bound and insoluble glucose at 15 and 45 min. The CP formed significantly more ICP than NCP at 15 and 45 min ($P < 0.001$, Wilcoxon signed rank test; Fig. 6). After the plaque cells metabolized ICP for 6 h, there was no significant difference in ICP levels between the plaque types (Fig. 6). The magnitude of ICP catabolism was therefore greater in the CP.

ECG formation. In the CP, 19% of the total cell-bound and insoluble glucose was ECG at 15 min and 23% was ECG at 45 min. In the NCP, ECG comprised 18% of the total cell-bound and insoluble glucose at 15 and 45 min. CP formed significantly more ECG at 15 and 45 min than NCP ($P < 0.001$, Wilcoxon signed rank test; Fig. 7). Any insoluble extracellular fructan formed by the plaque specimens during the incubation period would be lost during the hydrolysis procedure (40). The fructose levels in the plaque hydrolysates were monitored before

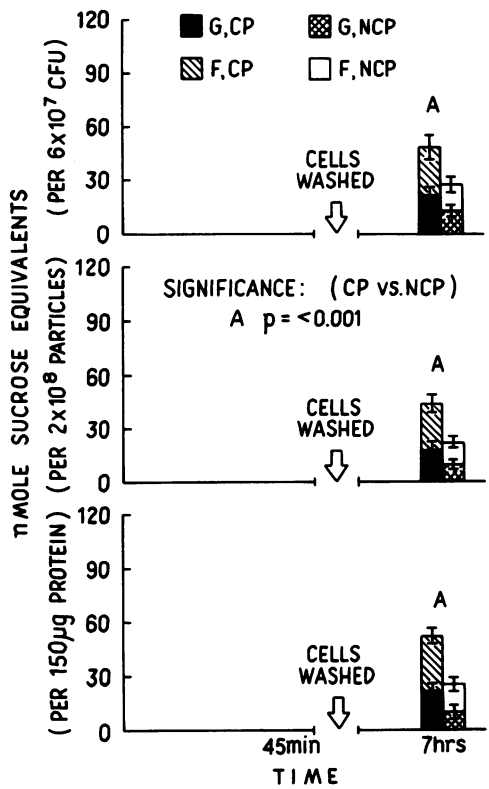

FIG. 5. *Lactic acid released from CP and NCP during ICP catabolism. Portions of resting-cell suspensions of dental plaque were filtered. The filters with retentates were incubated for 6 h at 37°C in RTF containing 2% sucrose and 2% sodium lactate. Lactic acid was isolated from samples of the incubation mixture by thin-layer chromatography and counted by liquid scintillation. Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each segment gives the average value and the brackets give the standard deviation. G = lactic acid derived from the glucose moiety of sucrose and F = lactic acid derived from the fructose moiety of sucrose. A = when the differences between CP and NCP were significant, $P < 0.01$.*

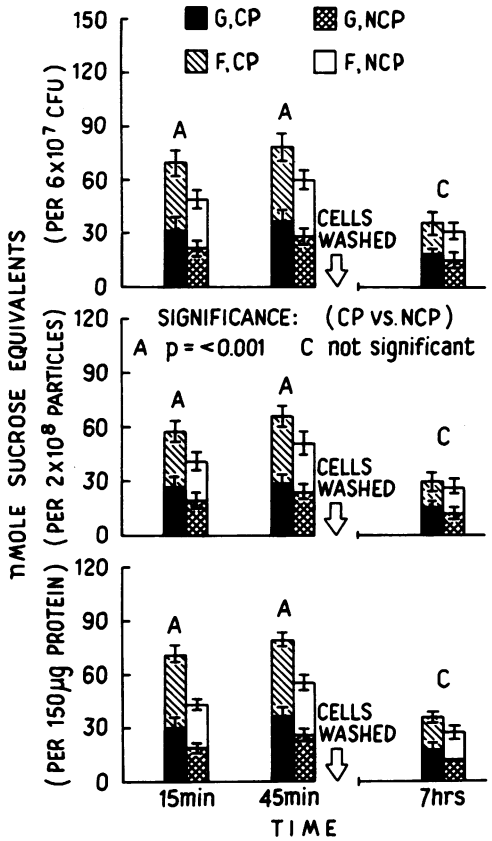

FIG. 6. *ICP formation and utilization in CP and NCP. Portions of resting-cell suspensions containing sucrose labeled with ^{14}C-fructose were filtered, and the retentates were exposed to Dowex 50W in 0.2 N HCl for 48 h at 100°C. Glucose in the hydrolysates was isolated by thin-layer chromatography and counted by liquid scintillation. ICP was calculated by the following formula: ICP = {1/[lactate from ICP(F)/lactate from ICP(G) + (F)]} × glucose (F) (ref). Bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each segment gives the mean and the brackets give the standard deviation. G = lactic acid derived from the glucose moiety of sucrose and F = lactic acid derived from the fructose moiety of sucrose. A = when differences between the CP and NCP were significant, P < 0.01; C = differences were not significant.*

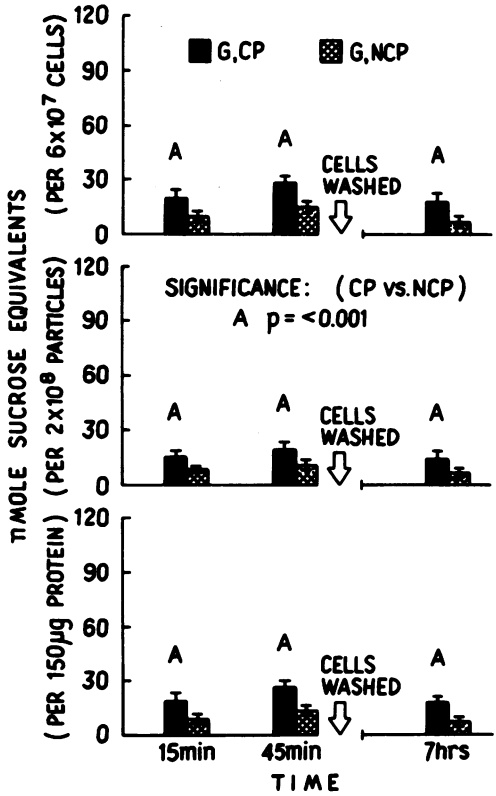

FIG. 7. *ECG formation in CP and NCP during 45 min of incubation. Portions of resting-cell suspensions were filtered, and the retentates were exposed to Dowex 50W in 0.2 N HCl for 48 h at 100°C. Glucose in the hydrolysates was isolated by thin-layer chromatography and counted by liquid scintillation. 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. The height of each segment gives the mean and the brackets give the standard deviation. G = ECG derived from the glucose moiety of sucrose; A = when differences between the CP and NCP were significant, P < 0.01.*

and after the second incubation (6 h) to assess labeled fructan utilization by the bacteria during the second incubation period. These fructose quantities were less than 1% of the consumed sucrose and did not significantly differ between the plaque types.

CO_2 formation. In both plaque types, CO_2 represented approximately 2% of the sucrose consumed after 45 min. The standard deviations ranged from 20 to 35% of the mean values. The CP formed significantly more CO_2 than the NCP ($P < 0.001$, Wilcoxon signed rank test). The fructosyl moiety of sucrose was favored in CO_2 production by both plaque types, as the G-to-F ratios for CO_2 were 0.72 in the CP and 0.57 in the NCP.

Total volatile acid production. Volatile acids represented 24% of the total sucrose consumed by the CP and 29% consumed by the NCP. The CP formed significantly more total volatile acids than the NCP when the specimens were quantitated by CFU and protein content ($P < 0.05$, Wilcoxon signed rank test; Fig. 8). The standard deviations ranged from 30

to 36% of the mean values. The glucosyl moiety of sucrose was favored by both plaque types in the formation of volatile acids, i.e., the G-to-F ratios were 1.2 in CP and 1.08 in NCP. Acetic acid accounted for 51% of the total volatile acids formed in the CP and 48% formed in the NCP. Propionic and butyric acids comprised 18 and 20% of the total volatile acids, respectively, in CP and 18 and 23%, respectively, in NCP. The differences in the proportions of acetic, propionic, and butyric acids by the two plaque types were not significant (Fig. 8). The CP, however, formed more of each of the volatile acids. The glucosyl moiety of sucrose was slightly favored by both plaque types in production of acetic and propionic acids. The G-to-F ratio in acetic acid was 1.1 in CP and 1.03 in NCP, and, in propionic acid this ratio was 1.2 in CP and 1.4 in NCP. Over half of the butyric acid formed in both plaque types was derived from the fructosyl moiety of sucrose; i.e., the G-to-F ratio was 0.9 in CP and 0.6 in NCP. Standard deviations as percentages of the means averaged from 28 to 36% for acetic acid, 27 to 34% for propionic acid, and 30 to 34% for butyric acid.

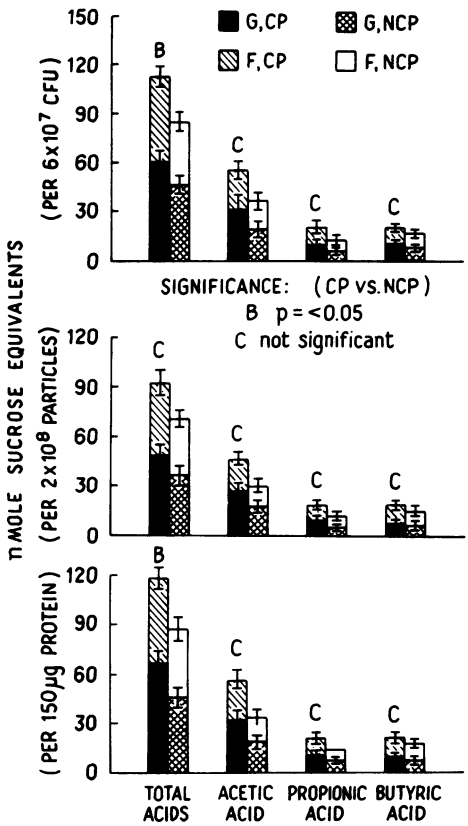

FIG. 8. *Volatile acid production in CP and NCP during 45 min of incubation. Total volatile acids were determined by a microdiffusion method. Individual volatile acids were determined by gas-liquid chromatography, using a beam splitter to capture the radioactive peaks. The bar values represent micromoles of sucrose converted to products as determined by recoveries of ^{14}C in the products. The height of each segment gives the mean and the brackets give the standard deviation. G = acids derived from the glucose moiety of sucrose and F = acids derived from the fructose moiety of sucrose. B = when differences between the CP and NCP were significant; C = when differences were not significant.*

The proportion of the consumed sucrose represented by the various metabolic by-products at each time period is summarized in Fig. 9. Lactic acid accounted for 36% of the sucrose consumed at each time period by the CP and for 30% of the sucrose consumed by the NCP. Both plaque types transformed approximately 43% of sucrose consumed at 15 min and 30% of the sucrose consumed at 45 min into cell-bound and insoluble products. In both plaque types the cell-bound and insoluble products represented a larger portion of the sucrose consumed at 15 min than at 45 min. ICP accounted for 61% of the cell-bound and insoluble product in the NCP and for 54% in the CP. ECG represented 18% of the total cell-bound and insoluble products in the CP and 14% in the NCP. The total volatile acids were derived from 24% of the sucrose consumed in the CP and 29% consumed in the NCP. Acetic acid accounted for 11% of the sucrose consumed in the CP and 12% consumed in the NCP. Propionic acid was formed from 4% of the sucrose consumed by the CP and 6% consumed by the NCP. Butyric acid resulted from 4% of the sucrose consumed by the CP and 3.5% consumed by the NCP. Soluble ECP was formed from 10% of the sucrose consumed in both plaque types. CO_2, free glucose, and free fructose together represented from 2 to 3% of the sucrose consumed by the plaque types.

DISCUSSION

The high levels of *S. mutans* in CP, i.e., 40% of the CFU and 75% of the streptococci, and low levels on noncarious surfaces confirmed earlier reports (5, 28–30, 32, 37, 38, 44, 45, 54). The consistent presence of *S. sanguis* in NCP and the infrequent presence in CP are in agreement with earlier reports (33, 38). The NCP harbored high levels of *Actinomyces* species (averaging 59% of CFU versus 24% in CP) and low levels of streptococci (3% of CFU versus 53% in CP). It is surprising that two sites separated by a few millimeters on the same tooth and exposed to exactly the same dietary substrates would be populated by such distinctly different levels of

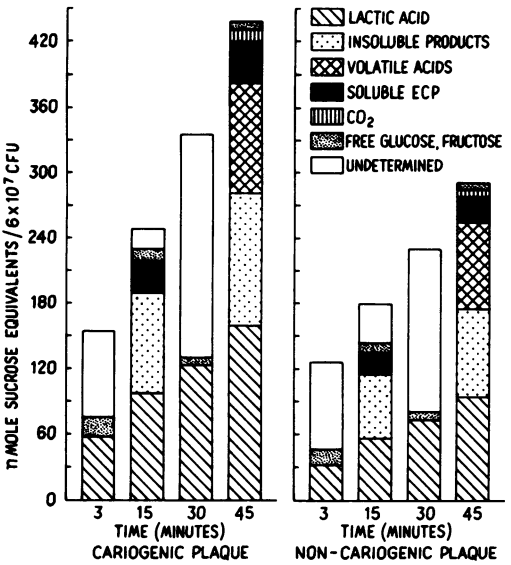

FIG. 9. *Distribution of by-products of sucrose metabolism in CP and NCP. The height of each bar represents the amount of sucrose consumed during the particular time interval. The height of each bar segment represents the proportion of the consumed sucrose recovered as the product indicated by the code on the figure.*

S. mutans. The possibility exists that certain bacteria in the NCP were antagonistic to *S. mutans*. *S. sanguis* appears to be the initial colonizer of the tooth surface (3, 52) and thereby might occupy space that otherwise could be colonized by *S. mutans*. As dextran formulation by *S. mutans* is important for its ascendancy in the plaque flora (8, 23, 24, 26), the presence of organisms such as actinomyces (46), which degrade dextran, might serve to keep the *S. mutans* levels low. In this regard, the NCP had significantly higher proportions of dextranase-producing organisms than did the CP.

The elevated levels of *Veillonella* sp. in the NCP could account for the slightly lower lactic acid values and slightly higher acetic and propionic acid values found in the NCP relative to the CP. As the volatile acids are weaker acids than lactate, this could minimize tooth decalcification. This last possibility has some experimental verification, as coinfection of gnotobiotic rats with *S. mutans* and *V. alcalescens* resulted in fewer carious lesions than were seen in rats that were monoinfected with *S. mutans* (39).

The metabolic experiments were designed to test current theories of cariogenesis. Most evidence suggests that acid production by bacteria, and lactic acid production, in particular, is involved in destruction of the dental enamel. The CP by this reasoning should form more lactic acid than NCP upon a short exposure to sucrose. This hypothesis was readily confirmed, as the rate of lactic acid formation was considerably higher in the CP than in the NCP (Fig. 2). Previous studies have found plaque from caries-active individuals to contain higher levels of iodophilic CFU than plaque from caries-inactive individuals (2, 18, 36). This suggested that the CP would contain or form more ICP than NCP, which indeed was the situation found (Fig. 6). Most of the ICP was formed within 15 min, and in the case of the CP this ICP accounted for up to 20% of the sucrose consumed in 15 min. The cariogenic factor associated with ICP formation is lactic acid produced during ICP catabolism. The accompanying low pH would provide a decalcifying environment at the tooth surface for several hours after the initial carbohydrate had been removed (18). The CP theoretically should form more acid during ICP catabolism than NCP, and this is what was found (Fig. 5).

The cariogenic effect of *S. mutans* ECG is well documented in animal experiments (8, 15, 16, 21, 23, 24, 26). In humans, CP with high levels of *S. mutans* should contain and form more ECG than NCP. This prediction was supported by the present findings, in which the CP formed about twice the ECG as did the NCP (Fig. 7). The ECG amounted to only 4 to 5% of the consumed sucrose in either plaque types. This agrees with previous reports which indicate that ECG comprises about 2 to 10% of the plaque weight (12, 41).

The rate of sucrose consumption during 45 min of incubation was noticeably different between the two types of plaque. The higher rate of sucrose consumption by the CP alone would explain the differences in levels of most of the metabolic by-products. The argument that this reflects differences in enzyme levels related to sucrose consumption would not apply, since both plaque types were exposed to the same dietary substrate. What it does imply is that CP contains bacterial types such as *S. mutans*, which are more efficient utilizers of sucrose than the actinomyces species that dominated in the NCP.

The overall rate of sucrose consumption by both CP and NCP, however, exhibited biphasic kinetics, being more rapid during the first 3 min than at the later time periods. This activity, which resembles the early rapid drop in pH and later gradual or negligible pH drop in in situ dental plaques (48) or pure cultures of plaque bacteria (49) after exposure to glucose, is not completely understood. One possible explanation is that the ICP levels of the bacteria

influence the rate of sucrose consumption. Since pure culture of oral bacteria exhibits biphasic kinetics in the rate of ICP synthesis (25), alterations in the rate of glycolysis in ICP formers would be expected. Another determining factor might be the loss of viability of the bacteria in the resting-cell suspensions with time (9). Finally, it is possible that the depletion of sucrose in the suspensions with time might retard the activity of enzymes, which maximally operate at higher sucrose concentrations.

The absolute quantity of cell-bound and insoluble products was higher in the CP owing to a faster sucrose consumption rate. The proportion of sucrose consumed that went into these cell-bound products in both plaque types declined with time. After 15 min of incubation, 43% of the sucrose consumed could be accounted for as cell-bound and insoluble polymers, whereas after 45 min only 30% of the consumed sucrose was represented in these polymers. This could mean that the rate of polymer synthesis decreased with time or that the polymers were degraded to acid and other products in the presence of substrate. This latter possibility is supported by evidence reported by Hamilton (25). Resting-cell suspensions of pure cultures of *S. sanguis*, *S. mutans*, and *S. salivarius* were able to degrade glycogen or ICP in the presence of exogenous glucose or sucrose. Although these observations do not preclude the important role of ICP as a reserve energy source, they suggest that the regulation of the degradative pathway is not controlled exclusively by the levels of the external carbon source. The fact that ICP is catabolized in the presence of sucrose made possible the detection of [^{14}C]lactic acid during the second incubation period (Fig. 5). These lactate values were essential for the calculation of ICP and ECG (41).

Volatile acids, especially acetic, accounted for a large proportion of the metabolic products in both plaque types (Fig. 9). These acids could be formed directly from sucrose or indirectly via lactic acid (10, 19, 42). The significance of volatile acids in cariogenesis is unclear. They possess a higher pK value than lactic acid, so that they are less likely to solubilize enamel, and their higher vapor pressure might enable them to escape from a plaque more readily than lactic acid. The CP formed more total volatile acids than NCP according to CFU and protein content. However, the NCP transformed proportionally more of the consumed sucrose into volatile acids than did CP.

The differences in formation of minor products such as CO_2, free glucose, and free fructose are difficult to assess in terms of cariogenesis. Free glucose and fructose levels were low and not significantly different in both plaque types. The significantly greater amounts of CO_2 found in the CP might reflect a greater breakdown of lactate to CO_2 and other products (10, 19, 42).

The preferential utilization of the two moieties of sucrose by the dental plaque bacteria is difficult to interpret in terms of cariogenic activity. Lactic acid, CO_2, soluble ECP, and ICP were derived to a slightly greater extent from the fructosyl moiety of sucrose, whereas volatile acids and free glucose were formed more readily from the glucosyl moiety. Tanzer et al. (50) reported that an *S. mutans* serotype *d* strain exposed to [^{14}C]sucrose uniformly labeled in the glucosyl moiety formed less labeled lactic acid than the same number of cells exposed to [^{14}C]sucrose uniformly labeled in the fructosyl moiety. The G-to-F ratio was 40 to 60. This finding resembles the G-to-F ratio in lactic acid in this investigation (i.e., the G-to-F ratio was 45 to 55 in both plaque types). There are at least two possible explanations for the preferential appearance of the fructosyl moiety of sucrose in lactic acid. The first explanation concerns glucosyltransferase activity by *S. mutans* and other plaque organisms which would release free fructose into the medium. If glucosyl transferases were more abundant in plaque than fructosyl transferases a greater net concentration of free fructose would be available for glycolysis. The second possibility is a corollary to the above, in that free fructose may act as an inhibitor of ECG synthesis (16). Thus, *S. mutans* and other organisms in plaque may preferentially remove fructose so that ECG synthesis may proceed at a maximal rate.

The metabolic experiments revealed clear differences between the plaque types, which coincided with the relative proportions and numbers of *S. mutans* in the plaques. Although this investigation does not prove that *S. mutans* initiates caries, it strongly implies that *S. mutans* is metabolically dominant in plaques associated with the carious lesion.

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