INFECTION AND IMMUNITY, Dec. 1978, p. 714-720
0019-9567/78/0022-0714$02.00/0

Vol. 22, No. 3
Printed in U.S.A.

Streptococcus mutans Dextransucrase: Stimulation by Phospholipids from Human Sera and Oral Fluids

CHARLES F. SCHACHTELE,* SUSAN K. HARLANDER, JAMES W. BRACKE, LEE C. OSTRUM, JO-ANN B. MALTAIS, AND RONALD J. BILLINGS

Microbiology Research Laboratories, School of Dentistry, and Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455

Received for publication 13 September 1978

Serum, gingival crevicular fluid, and parotid, submandibular, and labial minor gland saliva from four individuals stimulated glucan formation from sucrose by the *Streptococcus mutans* strain 6715 dextransucrase (EC 2.4.1.5). At final dilutions of 1:10 all of the fluids stimulated crude enzyme preparations approximately 1.8-fold. The fluids stimulated the purified water-insoluble glucan-synthesizing form of the dextransucrase approximately 3.2-fold and the water-soluble glucan-producing form of the enzyme approximately 2.4-fold. The fluids all contained concentrations of stimulatory material that could be reduced to undetectable levels only after dilutions of greater than 1:1,000. The increased rates of glucan formation caused by the fluids and dextran were additive, indicating that stimulation by the fluids was primarily due to interactions with entities other than glucan primer molecules. In contrast, the elevated levels of glucan formation in the presence of the fluids was not further enhanced by the addition of lysophosphatidylcholine. Lysophosphatidylcholine purified from parotid and submandibular saliva by solvent extraction and thin-layer chromatography stimulated the dextransucrase as effectively as egg yolk lysophosphatidylcholine. Thus, phospholipids normally found in human oral fluids can enhance the activity of an enzyme believed to be directly associated with the cariogenic potential of *S. mutans*.

Recent studies (17) have demonstrated that the *Streptococcus mutans* dextransucrase activity can be markedly stimulated by addition of phosphoglycerides. Enhanced glucan production results from an increase in the velocity of polymerization of the glucosyl moiety of sucrose due to a high affinity interaction between phospholipid and a site on the enzyme which appears to be distinct from either the glucosyl donor or glucosyl acceptor sites. We have proposed (28) that the observed stimulation of dextransucrase activity by control and/or immune sera and oral fluids from monkeys (27), rabbits (9, 10, 20, 22), and rats (4) is due to the presence of stimulatory phospholipids in these materials.

In this communication we demonstrate that dextransucrase-stimulating phospholipids are present in significant quantities in human sera, saliva from the parotid (PA), submandibular (SM) and minor salivary glands (LMG), and gingival crevicular fluid (GCF). The possible in vivo consequences of interactions between the *S. mutans* dextransucrase and oral fluids are discussed.

MATERIALS AND METHODS

Dextransucrase preparations. The different enzyme forms used in this study were from *S. mutans* strain 6715 (17). The preparation and characteristics of the crude Bio-Gel, water-soluble, and water-insoluble glucan-producing activities have been described (12).

Dextransucrase assay. The methods for quantitation of total alcohol-insoluble, water-soluble, and water-insoluble glucan production from [^{14}C]sucrose have been described previously (12, 14). Unless specified, primer dextran was present at a concentration of 20 μM. When test components were in a solvent other than 0.01 M sodium acetate (pH 5.5), appropriate solvent controls were utilized as described elsewhere (17).

Human sera. Blood was collected by venipuncture from laboratory personnel after overnight fasting. Clotting occurred in sterile tubes during overnight storage at 4°C. Serum was obtained after centrifugation (5,500 × *g*, 30 min) and was either utilized immediately in the enzyme studies or lyophilized and stored at 4°C.

Human saliva. PA saliva (5 to 10 ml) was obtained from four adults with a Teflon suction cup device patterned on the design of Shannon and Chauncey (31). Flow was stimulated with a sour candy, and the first milliliter of saliva was discarded to avoid possible bacterial contamination. Stimulated SM saliva was obtained by using a mouthpiece constructed for each individual with rubberbase impression material as described by Block and Brottman (2). Saliva from the

LMG of the same individuals was obtained as described by Crawford et al. (6). Fluid was obtained with 5-μl micropipettes and utilized immediately after an initial 10-fold dilution into 0.01 M sodium acetate (pH 5.5) buffer at 4°C. A portion of both the PA and SM saliva samples was immediately used in the enzyme studies, and the remainder was lyophilized for storage.

GCF. Ready access and ease of isolation were the primary considerations for choosing the facial and interproximal surfaces of the six maxillary anterior teeth as the sampling sites. The same individuals used to obtain saliva samples were asked to brush their teeth approximately 20 min before sampling to remove plaque. Brushing was not supervised; dentifrice was not used; and the subjects were asked not to brush vigorously to avoid excessive gingival irritation. The teeth were isolated and protected from salivary contamination by placing cotton rolls labially and gauze squares lingually, and by using a saliva aspirator. The teeth and gingivae were gently and thoroughly cleansed of saliva and any residual debris by using compressed air for 60 s. Whatman no. 1 filter paper strips (2 by 10 mm) were gently seated in the gingival crevice with Dixon no. 5 tweezers. Eight to ten strips were placed, with care taken to distribute the strips facially and interproximally. Facial strips, in general, were centered on the tooth at the site being sampled. Interproximal strips were placed mesial or distal to the labial gingival papilla. Care was taken to ensure that these strips were placed exclusively within the area of the labial gingival papilla. All of the sites tested were clinically normal according to the criteria of Loe (23). After 10 min, the strips were removed in the same order as placed. The length of migration of the absorbed fluid on each strip was quickly measured by vernier calipers. Estimation of the fluid collected per strip was made by comparing the length of migration on identical paper strips of a 1-μl sample of serum obtained from each individual. The estimated quantity of fluid collected on each strip was approximately 0.3 μl. The portion of the strips containing GCF was cut off and placed into a 400-μl plastic tube. A sufficient quantity of 0.05 M sodium acetate buffer (pH 5.5) was added to the tube containing all of the samples from each individual so that all of the strips were submerged. Extraction from the paper was performed by overnight storage at 4°C. The samples were gently mixed, and a portion of the fluid was used for further study.

Extraction of lipids. Lyophilized samples of sera and PA saliva were extracted by addition of chloroform–methanol (2:1, vol/vol) to a volume five times that of the original sample. After mixing and standing for 15 min at room temperature, the mixture was filtered through Whatman no. 1 filter paper. The residue remaining on the filter was extracted three times with 10 volumes of chloroform–methanol, and the pooled filtrates were taken to dryness under nitrogen. The total lipid fraction was treated with cold acetone to separate the neutral lipids and phospholipids (18). The efficiency of separation of the lipid classes was determined by adding 0.5 μCi of [4-^{14}C]cholesterol (54 mCi/mmol; New England Nuclear, Boston, Mass.) and 0.5 μCi of L-1-[palmitoyl-^{14}C]lysopalmitoyl phosphatidylcholine (55.3 mCi/mmol; New England Nuclear) to 1-ml samples of sera and PA saliva before extraction. Radioactivity in various fractions was determined by spotting duplicate 10-μl aliquots onto Whatman 3MM filter paper disks and counting the air-dried disks as described elsewhere (29).

Thin-layer chromatography. Glass plates (20 by 20 cm) coated with 250-μm Silica Gel H (Analabs, Inc., New Haven, Conn.) were activated at 110°C under vacuum for 30 min before each experiment. Samples were spotted on cooled plates under a continuous stream of dry nitrogen, using a Ziptrol applicator system (Bolab, Inc., Derry, N.H.). L-α-Lysophosphatidylcholine (LPC) from egg yolk, L-α-phosphatidylcholine (PC) from egg, L-α-phosphatidylinositol (PI) from soybean, L-α-phosphatidylserine (PS) from bovine brain, and L-α-phosphatidylethanolamine (PE) from egg were obtained from Avanti Biochemicals, Inc., Birmingham, Ala., and were used as standards. Plates were pre-equilibrated with a solvent system of chloroform–methanol–water (65:25:4, vol/vol) (21) for 35 min. Plates were either developed with phosphomolybdic acid spray reagent (Applied Science, State College, Pa.) or processed for stimulation assays. For the latter assays, the silica plate surface was scored with a needle to yield an area 0.5 cm wide extending from the origin of a sample lane distally to 2 mm beyond the solvent front of that sample lane. The plate was inverted over glassine weighing paper so that silica samples (0.5 cm by 1 mm) scraped from the lane would be retained on the paper. Samples were deposited into either glass scintillation vials for radioactivity measurements (27) or glass test tubes (10 by 75 mm). Control samples included two fractions taken from beyond the solvent front of the lane and from an artibrary area elsewhere on the plate that had been exposed to solvent but no sample material. Chloroform–methanol (2:1, vol/vol) was added (0.5 ml) to each fraction tube, blended in a Vortex mixer momentarily, and decanted into a fresh test tube. This elution procedure was repeated twice, with care taken not to decant silica fines. Fractions were maintained on ice under nitrogen. Eluent samples were dried under nitrogen immediately before enzyme stimulation studies.

RESULTS

Sera, oral fluids, and glucan synthesis. Addition of either human sera, PA, SM, or LMG saliva from all four subjects to a Bio-Gel *S. mutans* dextransucrase preparation caused a marked stimulation of glucan production from sucrose (Table 1). In comparison to the buffer control, the sera from the four subjects stimulated the enzyme approximately 1.8-fold. PA saliva enhanced glucan formation from 1.3-fold (subject A) to 1.6-fold (subject B). SM saliva stimulated the dextransucrase from 1.7-fold (subject D) to 1.9-fold (subject C), whereas the LMG saliva yielded comparable results. The level of stimulation of dextransucrase by the various fluids is similar to the effect observed by addition of pure LPC. As demonstrated previously, this phospholipid at 1 mM normally enhances glucan production by Bio-Gel *S. mutans* dextransucrase from 1.7-fold (17) to 1.8-fold (26).

The LPC and fluid effects were not additive since addition of the phosphoglyceride simultaneously with the sera or salivas did not cause stimulation above the level obtained with LPC alone (data not shown).

Table 2 illustrates the stimulation of dextransucrase by the addition of GCF collected with paper points and extracted from the paper with buffer. Although the final concentration of crevicular fluid components present in the assay tubes could not be accurately determined, the fluid collected from the four subjects when diluted approximately 1:10 caused a 1.6-fold (subject C) to 2.0-fold (subject A) increase in glucan formation. It is important that in all of these experiments (Tables 1 and 2) dextran T10 was present at a concentration previously shown to give maximum priming of the enzyme (11, 13).

Stimulation of different dextransucrase activities. Figure 1 illustrates the effect of the various fluids from one subject (B) on the time-course of total alcohol-insoluble glucan production by crude Bio-Gel dextransucrase (Fig. 1a), water-insoluble glucan formation by the α form of the enzyme (Fig. 1b), and water-soluble glucan production by the β form of the enzyme (Fig. 1c). All of the fluids markedly affected water-insoluble glucan production (Fig. 1b). After 60 min of incubation, the stimulation was approximately 3.2-fold with each of the fluids. The decrease or loss of accumulated glucan with the α enzyme was probably due to the dextranase activity previously shown to be present in this type of enzyme preparation (12). Water-soluble glucan production by the β enzyme was increased approximately 2.4-fold by both the sera and the different salivas. The stimulation in all cases is very similar to that obtained by addition of pure LPC to the reaction mixture (Fig. 1, closed squares).

TABLE 1. *Stimulation of crude S. mutans dextransucrase by serum and gland saliva from four individuals*

Assay conditions[a]	Glucan formed (nmol/μg of protein)[b] by subject:			
	A	B	C	D
Control (buffer)	84.1	84.1	84.1	84.1
Plus serum	151.9	150.7	155.5	157.3
Plus PA saliva	112.2	135.4	134.5	119.5
Plus SM saliva	152.2	150.3	161.9	146.6
Plus LMG saliva	130.6	144.4	164.2	166.4
Plus LPC	161.0	161.0	161.0	161.0

[a] Enzyme was incubated at room temperature for 10 min in buffer or buffer plus various fluids before assay. Saliva and sera were present at a final dilution of 1:10. LPC was present at a concentration of 1 mM.

[b] Bio-Gel enzyme was present at a final concentration of 20 μg of protein/ml, and the quantity of glucan present was determined after 60 min of incubation with substrate. Dextran T10 was present at a concentration of 20 μM.

TABLE 2. *Stimulation of crude S. mutans dextransucrase by GCF*

Assay conditions[a]	Glucan formed (nmol/μg of protein)[b] by subject:			
	A	B	C	D
Control	70.3	70.3	70.3	70.3
Plus GCF	139.6	127.0	113.7	138.5

[a] GCF, collected as described in Materials and Methods, was incubated with enzyme (1:1, vol/vol) for 10 min at room temperature before addition of reaction mixture. The final dilution of crevicular fluid was 1:10.

[b] Bio-Gel enzyme was assayed as described in Table 1.

Primer dextran and stimulation by sera and salivas. Table 3 presents data which confirm that the major portion of the stimulation of glucan production by the various fluids is probably not due to the supplying of primer dextran molecules to the dextransucrase. When dextran primer and serum or saliva are both present in the assay, the level of enzyme stimulation is approximately equivalent to the sum of the activity with either dextran or fluid alone. The results with LMG saliva demonstrate this most clearly in that the amount of glucan formed with dextran and saliva (128.9 nmol) is almost identical to the sum of the quantities of glucan formed with dextran and fluid alone (131 nmol). It was consistently observed that both PA and SM saliva caused lower levels of glucan to be made than expected from the studies with each saliva and dextran alone. In contrast, GCF caused production of more glucan than expected when added along with primer dextran. These results are consistent with those presented previously (17), where it was demonstrated that the stimulations of dextransucrase by LPC and dextran were additive.

Fluid dilutions and enzyme stimulation. Table 4 demonstrates that at the concentration of enzyme employed in our standard assay the glandular salivas and GCF had to be extensively diluted to remove the stimulating effect. Both PA and SM saliva had to be diluted 1:1,000, whereas LMG saliva still gave stimulation at dilutions 1:1,000 and 1:10,000. GCF had to be diluted more than 1:1,000 to eliminate the stimulation of dextransucrase. Although only the results of one subject's fluids are presented, the fluids from the other three subjects gave comparable results (data not shown).

LPC from saliva. Table 5 demonstrates the efficiency of separation by differential solvent

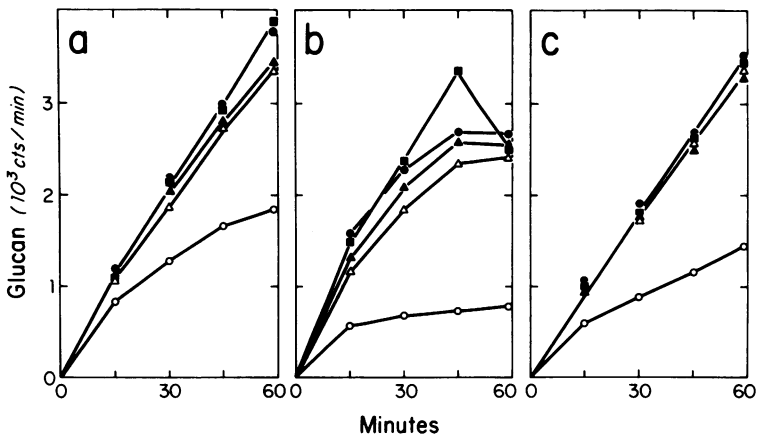

FIG. 1. *Effect of serum, saliva, and LPC on the time-course of glucan production by the S. mutans Bio-Gel (a), α (b), and β (c) enzyme preparations. PA saliva (△), SM saliva (▲), and serum (●) were present at a final dilution of 1:10. LPC (■) was present at a concentration of 1 mM. Buffer was added in the control assay (○). The Bio-Gel, α, and β enzymes were present at 20, 20, and 12 μg/ml, respectively.*

TABLE 3. *Effect of human oral fluids and primer dextran on stimulation of S. mutans dextransucrase*

Additions[a]	Glucan formed (nmol/μg of protein)[b]
Buffer	25.6
Dextran	82.9
PA saliva	51.0
Dextran plus PA saliva	96.3
SM saliva	67.2
Dextran plus SM saliva	130.1
LMG saliva	48.1
Dextran plus LMG saliva	128.9
GCF	38.6
Dextran plus GCF	145.9

[a] Enzyme was incubated for 10 min at room temperature in buffer or buffer plus T10 primer dextran (20 μM) before addition of various fluids. An additional 10-min incubation was performed before addition of reaction mixture.

[b] Bio-Gel enzyme was assayed as described in Table 1.

TABLE 4. *Effect of dilutions of human oral fluids on stimulation of S. mutans dextransucrase*

Final dilution[a]	Glucan formed (nmol/μg of protein)[b] from type of fluid			
	PA	SM	LMG	GCF
Control	66.0	64.5	44.6	57.8
10^{-1}	117.1	119.1	150.0	105.1
10^{-2}	97.4	73.2	84.9	67.8
10^{-3}	60.7	66.9	71.6	65.9
10^{-4}	62.7	65.7	52.7	60.8

[a] Fluids were diluted in 0.05 M sodium acetate buffer (pH 5.5).

[b] Enzyme preparation and assay conditions were as described for Table 1.

TABLE 5. *Efficiency of separation of neutral lipids and phospholipids from the total lipid fraction of human sera and PA saliva*

Sample[a]	Radioactivity (total cpm/fraction)	
	Cold acetone	Chloroform-methanol
Serum plus [^{14}C]cholesterol	760,460	4,150
Serum plus [^{14}C]LPC	8,940	441,680
PA saliva plus [^{14}C]cholesterol	752,435	1,650
PA saliva plus [^{14}C]LPC	5,380	218,080

[a] [^{14}C]cholesterol (0.5 μCi) and [^{14}C]LPC (0.5 μCi) were added to 1-ml portions of sera and saliva and the neutral lipids (cold acetone) and phospholipids (chloroform-methanol) were obtained as described in Materials and Methods.

extraction of neutral lipids and phospholipids from both human serum and PA saliva. The cold-acetone step clearly removed the neutral lipids from the total lipid fraction, and the chloroform-methanol fraction contained the phospholipids. Thin-layer chromatography of the phospholipid extracted from PA and SM saliva by this method allowed the preparation of LPC, which, when extracted from the silica gel, was shown to stimulate dextransucrase as effectively as egg yolk LPC (Table 6). Control thin-layer chromatography experiments with [^{14}C]LPC (Fig. 2, lower graph) and nonradioactive egg yolk LPC (Fig. 2, upper graph) demonstrated separation of LPC from other phospholipids (PC, PI, PS, and PE) and the ability of LPC-containing fractions to stimulate glucan formation after extraction from the silica gel.

TABLE 6. *Effect of LPC purified by thin-layer chromatography from human PA and SM saliva on the S. mutans dextransucrase*

Assay conditions[a]	Glucan formed (nmol/μg of protein)[b]
Control (Silica Gel H)	76.5
Plus egg yolk LPC	149.0
Plus PA saliva LPC	142.1
Plus SM saliva LPC	152.9

[a] Enzyme was incubated at room temperature for 10 min with the various additions. Egg yolk LPC was at a final concentration of 1 mM. The PA and SM saliva LPC samples were at a concentration equivalent to the amount extracted and purified from 4 ml of saliva under the conditions described in Materials and Methods.

[b] Bio-Gel enzyme was assayed as described in Table 1.

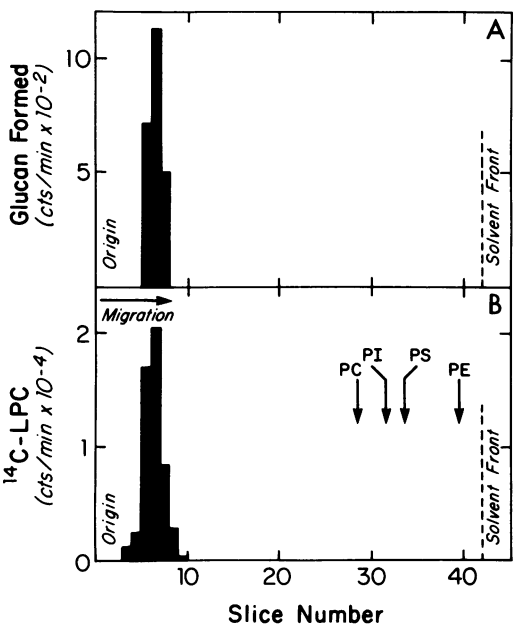

FIG. 2. *Thin layer chromatography of phosphoglycerides. (A) Silica gel fractions were obtained after chromatography of egg yolk LPC and extracted for phospholipid. The extracts were tested for their ability to stimulate glucan formation by the S. mutans dextransucrase. Shaded area represents the radioactivity incorporated into glucan over the silica gel control level. (B) Localization of radioactive LPC (shaded area) and control phosphoglycerides PC, PI, PS, and PE, which were detected with the phosphomolybdic acid spray reagent.*

DISCUSSION

During many studies designed to measure antibodies directed against *S. mutans* dextransucrase preparations, it has been consistently observed that control and sometimes immune sera caused an elevation rather than an inhibition of glucan production from sucrose (4, 9, 10, 20, 22, 27). Based on studies with sera and oral fluids from rats, Burckhardt and Guggenheim (4) proposed that there were sites on the *S. mutans* dextransucrase which could interact with molecules in the fluids and enhance glucan production. Our observations with pure phosphoglycerides (17) indicated that the *S. mutans* enzyme did contain such sites and that phospholipids could function as effective stimulators of glucan synthesis by a mechanism unique from dextran priming. We subsequently utilized these results (28) to develop a method where the addition of LPC and dextran to dextransucrase before addition of immune rabbit sera allowed measurement of antibody inhibition under conditions where the stimulating sites on the enzyme were already saturated. In addition, preliminary results (28) indicated that human serum contained high concentrations of stimulatory substances which could be obtained by lipid extraction. In the present study we have analyzed human sera and oral fluids to evaluate the presence and effectiveness of stimulatory phospholipids in these fluids.

Addition of freshly collected gland salivas (Table 1) and GCF (Table 2) to the *S. mutans* dextransucrase caused stimulation of glucan formation to a level comparable to that obtained with LPC (Fig. 1). The mechanism of stimulation appeared to be similar to that obtained with LPC (Table 3), since the degree of stimulation was unrelated to the presence of primer dextran. In addition, the quantity of stimulatory material in the oral fluids was high (Table 4), and the phosphoglyceride LPC, when extracted (Table 5) and purified (Fig. 2) from saliva, was shown to enhance glucan production (Table 6).

Although few detailed analyses have been presented, there is some information available concerning the lipid content of human saliva (7, 19, 24, 26, 32, 33). Rabinowitz and Shannon (26) have analyzed human male PA saliva and demonstrated that unstimulated saliva contained about 7 mg of lipid per 100 ml of fluid. Of the total lipid, approximately 24% was phosphoglycerides (PC, PI, PS, PE, and LPC) previously shown to stimulate the *S. mutans* dextransucrase (17). The concentrations of these phosphoglycerides was about 1.4 mg/100 ml of PA saliva. Since our assay system is capable of detecting stimulation of dextransucrase by as little as 10 μM LPC (17), the stimulation by PA saliva observed in our studies (Tables 1 and 4) was a reasonable finding. At this time we have no information concerning the stimulation of dextransucrase by any of the more complex lipid-

containing components in human saliva (32, 33), although the dilution data in Table 4 indicate that the various gland salivas contain either high levels of available phosphoglycerides or other molecules with stimulatory potential. The level of stimulatory lipids would be expected to vary with flow rate (31) and apparently with the gland secreting the fluid (Table 4).

Human sera contain phospholipids capable of stimulating dextransucrase (28; Table 1). It has been demonstrated that the concentration of phospholipid in sera is variable between subjects (1, 25) and that the level of LPC in sera is usually close to 0.2 mM (34). This concentration of LPC is about 20-fold higher than the amount needed to cause maximal stimulation of dextransucrase under our assay conditions (17). The serum dilution study presented previously (28) is consistent with these observations in that dilutions greater than 1:1,000 were necessary to reduce enzyme stimulation to an undetectable level.

Depending upon the disease state of the periodontium, GCF has been considered as either an altered serum transudate or an inflammatory exudate (15). We collected fluid from the gingival crevice of adults with healthy gingivae and obtained enough material to demonstrate the presence of stimulatory components (Tables 2 and 4). Our limited data are consistent with the indications that the composition of GCF is qualitatively similar to serum (3, 5, 8, 30). Although further work with GCF is necessary, it is likely that the components in GCF which affect glucan formation are phospholipids.

The immediate significance of the results presented in this manuscript is that we have been able to demonstrate that oral fluids have the ability to affect the activity of an enzyme believed to be critically related to the cariogenicity of *S. mutans*. As discussed previously (28), mutants of *S. mutans* that produce elevated or diminished levels of dextransucrase activity have been shown to have correspondingly increased or decreased cariogenicity when tested in animal models. It has been shown in many studies (see reference 16) that the metabolic activity of microorganisms cultured in vitro does not necessarily reflect that occurring in vivo. Based on our studies with phospholipids and the *S. mutans* dextransucrase, it can be proposed that, in the oral cavity, the ability of *S. mutans* to produce glucans from sucrose may be greater than envisioned from in vitro studies and that interactions with appropriate oral fluids either in saliva or within plaque may influence the survival and cariogenicity of this bacterium.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant DE 03654 from the National Institute of Dental Research. J.W.B., J.B.M., and R.J.B. were supported by Institutional National Postdoctoral Research Service Award DE 07014 from the National Institute of Dental Research.

LITERATURE CITED

1. **Berlin, R., C. O. Oldfelt, and O. Vikrot.** 1969. Lipid changes after incubation of normal plasma. Acta Med. Scand. **185**:427–431.
2. **Block, P. L., and S. Brottman.** 1962. A method of submaxillary saliva collection without cannulization. N. Y. State Dent. J. **28**:116–118.
3. **Brandtzaeg, P.** 1965. Immunochemical comparison of protein in human gingival pocket fluid, serums and saliva. Arch. Oral Biol. **10**:759–803.
4. **Burckhardt, J. J., and B. Guggenheim.** 1976. Interactions of antisera, sera, and oral fluid with glucosyltransferase. Infect. Immun. **13**:1009–1022.
5. **Courts, F. J., R. J. Boackle, H. H. Fudenberg, and M. S. Silverman.** 1977. Detection of functional complement components in gingival crevicular fluid from humans with periodontal disease. J. Dent. Res. **56**: 327–331.
6. **Crawford, J. M., M. A. Taubman, and D. J. Smith.** 1975. Minor salivary glands as a major source of secretory immunoglobulin A in the human oral cavity. Science **190**:1206–1209.
7. **Dirksen, T. R.** 1963. Lipid constituents of whole and parotid saliva. J. Dent. Res. **42**:920–924.
8. **Egelberg, J.** 1969. Cellular elements in gingival pocket fluid. Acta Odont. Scand. **21**:283–287.
9. **Evans, R. T., and R. J. Genco.** 1973. Inhibition of glucosyltransferase activity by antisera to known serotypes of *Streptococcus mutans*. Infect. Immun. **7**: 237–241.
10. **Fukui, K. Y., Y. Fukui, and T. Moriyama.** 1974. Some immunochemical properties of dextransucrase and invertase from *Streptococcus mutans*. Infect. Immun. **10**: 985–990.
11. **Germaine, G. R., A. M. Chludzinski, and C. F. Schachtele.** 1974. *Streptococcus mutans* dextransucrase: requirement for primer dextran. J. Bacteriol. **120**: 287–294.
12. **Germaine, G. R., S. K. Harlander, W. S. Leung, and C. F. Schachtele.** 1977. *Streptococcus mutans* dextransucrase: functioning of primer dextran and endogenous dextranase in water-soluble and water-insoluble glucan synthesis. Infect. Immun. **16**:637–648.
13. **Germaine, G. R., and C. F. Schachtele.** 1976. *Streptococcus mutans* dextransucrase: mode of interaction with high-molecular-weight dextran and role in cellular aggregation. Infect. Immun. **13**:365–372.
14. **Germaine, G. R., C. F. Schachtele, and A. M. Chludzinski.** 1974. Rapid filter paper assay for the dextransucrase activity from *Streptococcus mutans*. J. Dent. Res. **53**:1355–1360.
15. **Golub, L. M., and I. Kleinberg.** 1976. Gingival crevicular fluid: a new diagnostic aid in managing the periodontal patient, p. 49–61. *In* A. H. Melcher and S. A. Zarg (ed.), Oral sciences reviews, vol. 8. Munksgaard, Copenhagen.
16. **Gordon, D. F., and R. J. Gibbons.** 1967. Glycolytic activity of *Streptococcus mitis* grown in vitro and in gnotobiotic animals. J. Bacteriol. **93**:1735–1736.
17. **Harlander, S. K., and C. F. Schachtele.** 1978. *Streptococcus mutans* dextransucrase: stimulation of glucan formation by phosphoglycerides. Infect. Immun. **19**: 450–461.
18. **Kates, M.** 1972. Techniques of lipidology, p. 393–394. *In* T. S. Work and E. Work (ed.), Laboratory techniques in biochemistry and molecular biology. American Elsevier Publishing Co., Inc., New York.
19. **Krasnow, F.** 1945. Physiological significance of phospholipid in human saliva. J. Dent. Res. **24**:319–326.
20. **Kuramitsu, H. K., and L. Ingersoll.** 1976. Differential inhibition of *Streptococcus mutans* in vitro adherence

by anti-glucosyltransferase antibodies. Infect. Immun. **13**:1775–1777.

21. **Lepage, M.** 1964. Isolation and characterization of an esterified form of steryl glucoside. J. Lipid Res. **5**: 587–592.
22. **Linzer, R., and H. D. Slade.** 1976. Characterization of an antiglucosyltransferase serum specific for insoluble glucan synthesis by *Streptococcus mutans*. Infect. Immun. **13**:494–500.
23. **Loe, H.** 1967. The gingival index, the plaque index and the retention index system. J. Periodontol. **38**:610–616.
24. **Mandel, I. D., and A. Eisenstein.** 1969. Lipids in human salivary secretions and salivary calculus. Arch. Oral. Biol. **14**:231–233.
25. **Nicholls, D. G., J. R. Hampton, and J. R. A. Mitchell.** 1971. Cyclical changes in plasma-lysolecithin induced by oral contraceptives. Lancet **ii**:1428–1429.
26. **Rabinowitz, J. L., and I. L. Shannon.** 1975. Lipid changes in human male parotid saliva by stimulation. Arch. Oral Biol. **20**:403–406.
27. **Russell, M. W., S. J. Challacombe, and R. Lehner.** 1976. Serum glycosyltransferase-inhibiting antibodies and dental caries in Rhesus monkeys immunized against *Streptococcus mutans*. Immunology **30**:619–627.
28. **Schachtele, C. F., S. K. Harlander, L. C. Ostrum, J. W. Bracke, and J. L. Babb.** 1978. *Streptococcus mutans* dextransucrase: phosphoglycerides and the detection of inhibitory antibodies in sera. Adv. Exp. Med. Biol. **107**:717–726.
29. **Schachtele, C. F., A. E. Loken, and D. J. Knudson.** 1972. Preferential utilization of the glucosyl moiety of sucrose by a cariogenic strain of *Streptococcus mutans*. Infect. Immun. **5**:531–536.
30. **Schenkein, H. A., and R. J. Genco.** 1977. Gingival fluid and serum in periodontal disease. I. Quantitative study of immunoglobulins, complement components, and other plasma protein. J. Periodontol. **48**:772–777.
31. **Shannon, I. L., and H. H. Chauncey.** 1967. A parotid fluid collection device with improved stability characteristics. J. Oral Ther. Pharmacol. **4**:93–97.
32. **Slomiany, B. L., and A. Slomiany.** 1977. Partial characterization of glyceroglucolipids from human saliva. Biochem. Biophys. Res. Commun. **79**:61–66.
33. **Slomiany, B. L., and A. Slomiany.** 1978. ABH-blood-group antigens and glycolipids of human saliva. Eur. J. Biochem. **85**:249–254.
34. **Vikrot, O.** 1964. Quantitative determination of plasma phospholipids in pregnant and non-pregnant women, with special reference to lysolecithin. Acta Med. Scand. **175**:443–453.