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Effect of Human Saliva on Glucose Uptake by *Streptococcus mutans* and Other Oral Microorganisms

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We examined the effects of human whole salivary supernatant and parotid fluid on glucose uptake by *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Actinomyces viscosus*, *Staphylococcus aureus*, and *Escherichia coli*. The following three effects of saliva were observed: (i) inhibition of glucose uptake (*S. mutans*, *S. sanguis*), (ii) promotion of a transient, rapid (0 to 30 s) burst of glucose uptake (*S. mutans*, *S. sanguis*), and (iii) enhancement of glucose uptake (*S. mitis*, *A. viscosus*, *S. aureus*, *E. coli*). We observed no differences between the effects of whole salivary supernatant and the effects of parotid fluid. Heat treatment (80°C, 10 min) of saliva or the addition of dithiothreitol abolished inhibition of glucose uptake. Supplementation of saliva with H_2O_2 potentiated inhibition of glucose uptake. *S. mitis* and *A. viscosus*, which were stimulated by saliva alone, were inhibited by H_2O_2-supplemented saliva; 50% inhibition of glucose uptake by *S. mutans* and *S. mitis* required ca. 10 μM H_2O_2 in 50% (vol/vol) saliva. Loss of the inhibitory action of saliva occurred at about 5% (vol/vol) saliva. Supplementation of saliva dilutions with SCN^- and H_2O_2 extended the inhibitory activity to solutions containing ca. 0.2% (vol/vol) saliva. We suggest that the salivary lactoperoxidase-SCN^--H_2O_2 system is responsible for the inhibitory activity of saliva reported here. Furthermore, we concluded that lactoperoxidase and SCN^- are present in saliva specimens in concentrations that exceed minimal inhibitory levels by factors of ca. 500 and 10 to 20, respectively. The resistance of *A. viscosus*, *S. aureus*, and *E. coli* to the inhibitory potential of saliva alone was probably due to the production of catalase by these organisms. The resistance of *S. mitis* may have been due to special effects of saliva on H_2O_2 accumulation by this organism compared with *S. mutans* and *S. sanguis*. The basis of saliva-dependent enhancement of glucose uptake and the basis of promotion of a transient, rapid burst of glucose uptake are unknown. The role of the salivary lactoperoxidase-SCN^--H_2O_2 system in the oral microbial ecosystem is discussed.

Saliva is the principal fluid that bathes the oral tissues and is a dominant factor in the maintenance of a stable oral microbial ecology and, therefore, oral health (6, 24, 25). The loss of or a marked reduction in saliva flow is accompanied by major changes in the composition of the oral flora (7, 9, 22, 23). Saliva and its constituents may inhibit or enhance bacterial growth and fermentation (6, 15, 19, 24), promote lysis of bacteria (31), and inhibit or enhance bacterial adherence to oral tissues (12, 47, 50, 51). Several substances with potential antimicrobial activity are normal constituents of saliva. These include lactoperoxidase, lysozyme, lactoferrin, mucins, immunoglobulins, and small cationic proteins (5, 24, 42). Saliva also contains substrates for growth of bacteria and additional constituents that may actually promote bacterial growth (19). Therefore, saliva may be viewed as an ambivalent fluid, a fluid that may promote the intraoral establishment of some microorganisms and discourage the establishment of others. Bacteria that are promoted by saliva may also play a crucial role in maintaining a stable flora through interbacterial effects (35, 40).

Saliva contains several substances that may potentiate or inhibit the activity of individual antimicrobial constituents. For example, it has been suggested that lysozyme activity might be enhanced in situ by lactoferrin through binding of inhibitory iron or copper (26, 49) and by salivary thiocyanate ion (SCN^-), which promotes lysis of streptococci (31). On the other hand, salivary mucins inhibit lysozyme activity (38, 48). Other examples include catalase interference with the lactoperoxidase system (20, 28) and immunoglobulin A-lysozyme interactions (11, 24). Most studies of salivary antibacterial

substances have used partially purified, reconstituted systems. These studies have contributed greatly to our understanding of the mode(s) of action of these systems. Although it is clear that salivary secretions contain both potentially enhancing and potentially inhibiting factors, it is difficult to assess the net effect which saliva might have on a microbial process if fractionated or reconstituted saliva is used. In an effort to develop systems which may approach in vivo conditions more closely, we initiated studies of bacteria suspended in unfractionated saliva specimens. Presumably, under these conditions interactions among salivary constituents and interactions between these constituents and the bacteria may occur such that the net effects of saliva may be observed. In this paper we describe the effects of unfractionated whole salivary supernatant and parotid fluid on glucose uptake by several oral microorganisms.

MATERIALS AND METHODS

Bacteria. The bacteria used included *Streptococcus mutans* BHT, FA-1, and Ingbritt, *Streptococcus mitis* 9811, *Streptococcus sanguis* S7 and 903, *Actinomyces viscosus* T6, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. All microorganisms were grown in Trypticase soy broth containing 0.5% yeast extract and 0.25% glucose. The streptococci were incubated in an anaerobic atmosphere (GasPak; BBL Microbiology Systems, Cockeysville, Md.), and *A. viscosus* was incubated in a CO_2-enriched atmosphere (GasPak). *S. aureus* and *E. coli* were grown aerobically. Bacteria were harvested in late log to early stationary phase (absorbance at 540 nm of 0.6 to 1.2) by centrifugation, washed twice with 50 mM potassium phosphate buffer (pH 7.0), and resuspended in the same buffer to yield an absorbance of 1.0.

Saliva. Collection of unstimulated whole saliva and collection of stimulated parotid fluid have been described previously (11, 39). Salivary supernatants were kept in an ice bath until used (less than 1 h). All saliva used in this study was freshly collected. The lactoperoxidase activity of saliva was estimated as described previously (14). Salivary thiocyanate was estimated as described elsewhere (2). Initial experiments demonstrated that saliva did not interfere with SCN^- determinations. Thus, the addition of SCN^- to saliva yielded a standard curve parallel (but elevated) to the curve obtained with SCN^- alone. Dialysis of saliva removed endogenous SCN^-, and upon readdition of SCN^- we obtained a dose-response curve that was superimposed on the primary SCN^- standard curve.

Glucose uptake. Suspensions of washed bacteria (absorbance, 1.0) in 50 mM phosphate buffer (pH 7.0) were preincubated for at least 30 min at 37°C (27). Bacteria were collected by centrifugation, resuspended in 0.5 volume of 50 mM phosphate buffer, and equilibrated to 37°C. For glucose uptake measurements, bacteria were preincubated (absorbance, 1.0) in 50 mM phosphate buffer containing any other desired compounds or saliva or both for 10 min at 37°C. Next, 0.85 volume of the preincubation mixture was added to 0.15 volume of prewarmed [U-^{14}C]glucose (1.5 mCi/mmol) to give a final concentration of 200 μM glucose, and incubation was continued at 37°C. With the exception of glucose, the quoted concentrations of all other compounds present in the uptake mixtures (see below) refer to the concentrations present during the preincubation (10-min) period. At the appropriate times 50- to 200-μl samples were removed for estimation of glucose uptake by the organisms. Two methods of sample treatment were used. The first method (27) employed membrane filtration (pore size, 0.45 μm) of samples, followed immediately by one wash with 10 ml of prewarmed (37°C) 50 mM phosphate buffer. Filter membranes were dried, and the amount of retained radioactivity was determined (10). The second method consisted of applying 50- to 100-μl samples to Whatman 3MM filter paper circles. These papers were then immediately immersed in methanol. At the end of an experiment the paper circles were batch washed in three changes of methanol and dried. Radioactivity levels were determined as described above. The second procedure for estimation of glucose uptake gave results equivalent to those of the membrane filtration procedure (a full description of the applicability of the Whatman 3MM filter paper procedure will be given elsewhere). Radioactive samples were counted to an error of 1 to 3%. Counting efficiencies were 60 and 80% for samples applied to Whatman 3MM filter papers and membrane filters, respectively.

RESULTS

Effect of saliva on glucose uptake. An examination of the uptake of glucose by whole-cell suspensions of several oral microorganisms and *E. coli* in the presence of 50% (vol/vol) whole salivary supernatant revealed that each microorganism consistently exhibited either an increased or a decreased rate of glucose uptake. Examples of the two responses are shown in Fig. 1. Both *S. mutans* BHT and *S. sanguis* 903 exhibited reduced rates of glucose uptake in the presence of saliva (Fig. 1a and b). *A. viscosus* T6 and *S. mitis* 9811 exhibited increased rates of glucose uptake in the presence of saliva (Fig. 1c and d). The effects of saliva on the rates of glucose uptake by several microorganisms are summarized in Table 1. Based on the limited number of strains tested, it appeared that *S. mutans* and *S. sanguis* were similar in that saliva diminished glucose uptake by these two microorganisms, whereas saliva stimulated glucose uptake by *S. mitis*, *A. viscosus*, *S. aureus*, and *E. coli*.

Figure 1a shows that the time course of glucose uptake in the presence of saliva did not pass through the origin. Although the rate was reduced compared with the buffer control rate, at the early sampling times (30 to 90 s) glucose uptake was actually greater in the presence of saliva. It seemed possible that saliva might cause either (i) an anomolous higher background re-

tention of glucose on filter membranes or Whatman 3MM filter papers, or (ii) a rapid burst of glucose uptake between 0 and 30 s, followed by a slower rate of uptake thereafter. In an effort to establish the basis of this phenomenon in saliva, glucose uptake by *S. mutans* BHT in buffer and in saliva was compared with uptake in a reaction mixture that contained only saliva and buffer (i.e., bacteria were omitted). Figure 2 shows that saliva alone did not give rise to an anomolous background retention of glucose. However, preincubation of *S. mutans* BHT in saliva containing 10 mM NaF resulted in (i) an 80% decrease in the rate of glucose uptake (compared with preincubation in saliva alone), and (ii) a time course of uptake that went through the origin (Fig. 3). This observation suggested that saliva may indeed cause a rapid initial burst of glucose uptake. In addition, since F^- inhibited the initial burst, this burst may depend on the phosphoenolpyruvate-dependent phosphotransferase system. Fluoride is an inhibitor of enolase, which generates phosphoenolpyruvate. Phosphoenolpyruvate is required for the transport of glucose via the glucose phosphotransferase system (21).

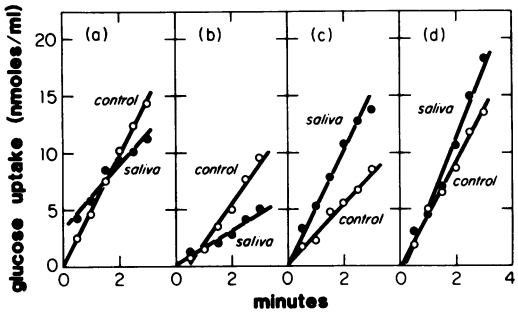

FIG. 1. *Glucose uptake in buffer and whole salivary supernatant. (a) S. mutans BHT. (b) S. sanguis 903. (c) A. viscosus T6. (d) S. mitis 9811. Glucose uptake by cells suspended in buffer* (○) *and 50% (vol/vol) saliva* (●).

TABLE 1. *Effect of saliva on glucose uptake by selected microorganisms*

Strain	Rate of glucose uptake (nmol/ml per min)[a]	
	Control[b]	Saliva[c]
S. mutans		
BHT	4.75	3.0 (63)[d]
Ingbritt	5.25	1.13 (22)
FA-1	17.5	4.0 (23)
S. sanguis		
S7	2.00	0.75 (38)
903	4.08	1.83 (45)
S. mitis 9811	4.50	6.00 (133)
A. viscosus T6	2.83	5.00 (177)
S. aureus	10.0	13.0 (130)
E. coli	22.7	24.6 (108)

[a] Determined from 0- to 2-min time course.
[b] The controls contained 50 mM potassium phosphate buffer alone.
[c] Saliva (50%, vol/vol) in 50 mM potassium phosphate buffer.
[d] Numbers in parentheses are percentages, which were determined as follows: [(rate in saliva)/(control rate)] × 100.

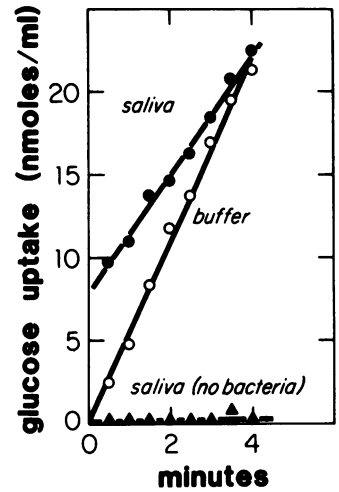

FIG. 2. *Glucose uptake by S. mutans BHT suspended in buffer or saliva. The line marked saliva (no bacteria) gives the results for a control uptake mixture that contained 50% (vol/vol) whole salivary supernatant and [^{14}C]glucose but no bacteria.*

The presence of 50% saliva in the preincubation mixture resulted in a decrease in the rate of glucose uptake by *S. mutans* of about 50%. In eight experiments, the rate of uptake in the presence of saliva was 53% (range, 45 to 69%) of the rate observed in buffer (data not shown). The effect of saliva concentration during preincubation with *S. mutans* BHT on the subsequent rate of glucose uptake was also examined (Fig. 4). Preincubation mixtures contained 0 to 80% (vol/vol) saliva. In the control (no saliva) the rate of uptake was 5.10 nmol/ml per min. Less than 2.5% saliva had no effect. When 2.5 to 20% saliva was present, the rate of glucose uptake decreased to ca. 50% of the control rate. Further increases in the saliva concentration up to 80% did not have any additional effect.

Basis of reduced glucose uptake in saliva. Previously, it had been reported that purified lactoperoxidase inhibited glucose transport in streptococci (27). Since saliva is also known to contain lactoperoxidase and SCN^- and since streptococci are known to produce H_2O_2, we investigated aspects of the saliva system that

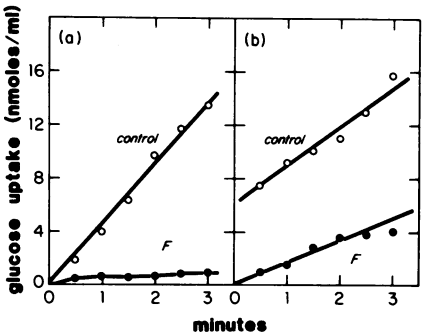

FIG. 3. *Effect of NaF on glucose uptake by S. mutans BHT. (a) Buffer-suspended cells. (b) Saliva-suspended cells. The control suspensions contained no NaF. Where indicated, NaF was present at a concentration of 10 mM.*

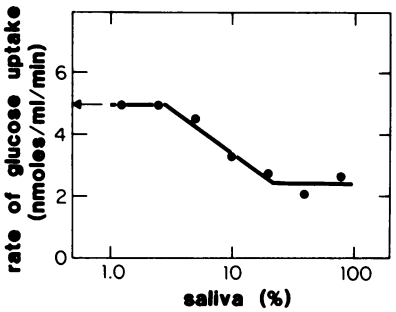

FIG. 4. *Effect of saliva concentration on glucose uptake by S. mutans BHT. The lactoperoxidase activity of undiluted saliva was 3.37 U/ml.*

might implicate lactoperoxidase in the inhibition of glucose uptake. Whole salivary supernatant heated to 80°C for 10 min failed to reduce the rate of glucose uptake significantly (91% of buffer control rate), whereas unheated saliva reduced the rate to 49% of the buffer rate (Fig. 5). Furthermore, the heated saliva promoted a greater initial burst of glucose uptake (an increase of ca. fourfold, as judged by the extrapolated intercept on the ordinate). A lactoperoxidase assay of unheated and heated saliva specimens indicated that more than 97% of the enzyme activity was destroyed by the heat treatment (unheated, 2.22 U/ml; heated, 0.05 U/ml). Therefore, inhibition of glucose uptake by saliva was dependent upon a heat-sensitive component. Supplementation of saliva with 100 μM H_2O_2 completely abolished glucose uptake (Fig. 5). In contrast, 100 μM H_2O_2 had no effect on buffer-suspended cells. Reducing agents, such as dithiothreitol (DTT) and glutathione, have been shown to inhibit lactoperoxidase-induced damage of cells and to reverse previous damage (18, 27, 33, 34, 46). Therefore, we examined the effect of supplementing saliva with 1 mM DTT. The presence of DTT had no effect on glucose uptake by buffer-suspended cells (Fig. 6). Saliva-suspended cells exhibited a rate of glucose uptake that was 48% of the rate in cells suspended in buffer. Glucose uptake in saliva supplemented with DTT was 104% of the rate in buffer. Thus, DTT abolished the inhibitory effect of saliva. Hydrogen peroxide potentiated the inhibition.

If lactoperoxidase is indeed the principal salivary constituent responsible for the inhibition of glucose uptake by *S. mutans* BHT, the lack of an inhibitory effect of saliva on glucose uptake by *A. viscosus*, *S. aureus*, and *E. coli* could be due to the lack of a source of H_2O_2, since these organisms are catalase producers. In the case of *S. mitis*, H_2O_2 production presumably was either absent or too low under our experimental con-

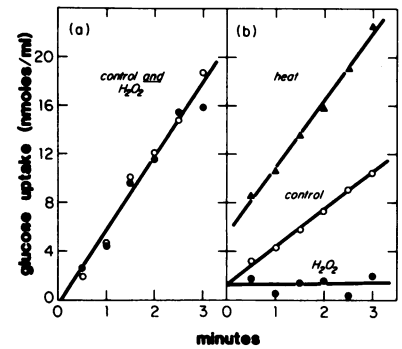

FIG. 5. *Effect of heat and H_2O_2 on glucose uptake by saliva-suspended S. mutans BHT. (a) Buffer-suspended cells. (b) Saliva (50%, vol/vol)-suspended cells. Control refers to no heat or H_2O_2. Saliva was heated for 10 min at 80°C. H_2O_2 was present at a concentration of 100 μM.*

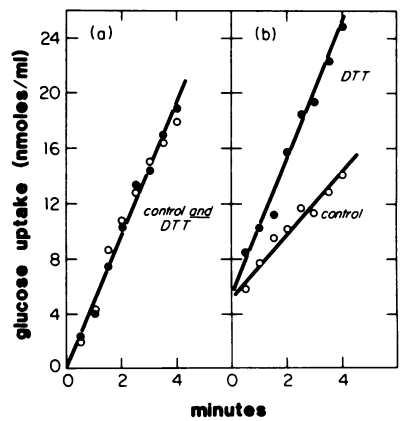

FIG. 6. *Effect of DTT on glucose uptake by S. mutans BHT. (a) Buffer-suspended cells. (b) Saliva-suspended cells. DTT was present at a concentration of 1 mM.*

ditions to allow sufficient damage to be produced by the lactoperoxidase system. Therefore, we investigated the effect of adding 100 μM H_2O_2 to preincubation mixtures of *S. mitis* containing buffer or saliva (Fig. 7). Hydrogen peroxide had no effect on cells in buffer. In contrast, the rate of glucose uptake by cells in saliva was reduced in the presence of H_2O_2 to only 20% of the rate observed in the presence of saliva alone. A similar observation was made with *A. viscosus*. H_2O_2 had no effect on buffer-suspended cells, but it reduced the rate of glucose uptake by saliva-suspended cells to 35% of the saliva control rate (data not shown). We also examined the effect of H_2O_2 concentration (1 to 100 μM) on the rate of glucose uptake by *S. mitis* suspended in saliva (Fig. 8). H_2O_2 at a concentration of 5 μM had little effect on glucose uptake (i.e., 90% of control rate), but 50% inhibition of glucose uptake occurred at ca. 10 μM H_2O_2. Inhibition was essentially complete at 50 μM H_2O_2.

In the case of *S. mutans* saliva alone (i.e., no added H_2O_2) reduced glucose uptake compared with uptake by buffer-suspended cells. Furthermore, 100 μM H_2O_2 caused total inhibition of glucose uptake by saliva-suspended cells. A more complete examination of the effect of different concentrations of H_2O_2 on glucose uptake by *S. mutans* indicated that an additional reduction of uptake to 50% of the rate observed with saliva alone required ca. 7 to 8 μM H_2O_2 (Fig. 8). At 20 and 100 μM H_2O_2 the rates of glucose uptake were reduced to 20 and 5% of the control rate, respectively. Furthermore, if heated saliva (80°C, 10 min) was used, 100 μM H_2O_2 had no effect on the rate of glucose uptake by *S. mutans* (Fig. 8).

Parotid fluid. Parotid fluid reproduced exactly the effects of whole salivary supernatant on glucose uptake by *S. mutans* and *S. mitis* (Fig. 9 and 10). Parotid fluid reduced glucose uptake by *S. mutans* more than 50% compared with the heated parotid fluid control. Furthermore, DTT abolished inhibition, and the addition of H_2O_2 resulted in complete inhibition of glucose uptake. In the case of *S. mitis*, parotid fluid stimulated glucose uptake compared with uptake by buffer-suspended cells. Also, an initial burst of glucose uptake was observed with *S. mutans* but not with *S. mitis*.

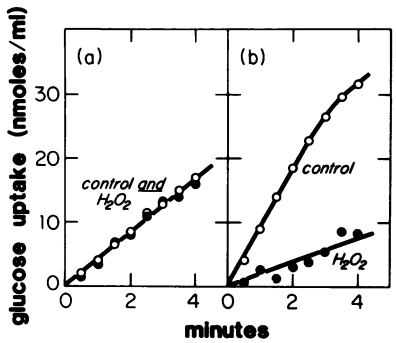

FIG. 7. *Effect of H_2O_2 on glucose uptake by S. mitis 9811. (a) Buffer-suspended cells. (b) Saliva (50%, vol/vol)-suspended cells. H_2O_2 was present at a concentration of 100 μM.*

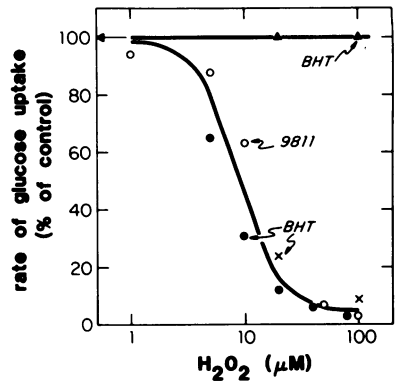

FIG. 8. *Effect of H_2O_2 concentration on glucose uptake by S. mutans BHT and S. mitis 9811. Cells were suspended in unheated (○, ●, ×) or heated (▲) saliva (50%, vol/vol). Heat treatment was for 10 min at 80°C. Unheated saliva contained 1.34 U of lactoperoxidase activity per ml.*

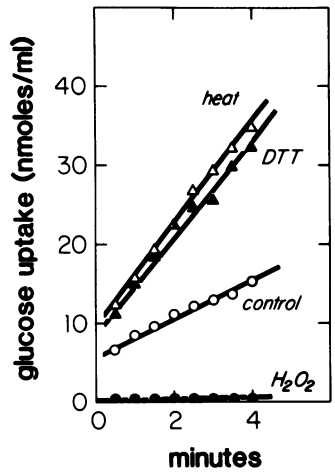

FIG. 9. *Effect of parotid fluid on glucose uptake by S. mutans BHT. Cells were suspended in 50% (vol/vol) parotid fluid alone (control) or with 100 μM H_2O_2 or 1 mM DTT. Heated parotid fluid was treated at 80°C for 10 min.*

Inhibitory potential of saliva. In an effort to estimate the biologically effective concentrations of the components of the salivary lactoperoxidase system, we examined the effect of saliva concentration on glucose uptake by *S. mutans*.

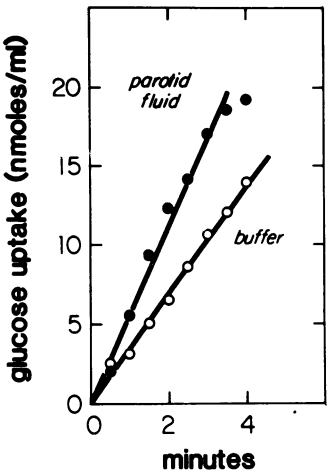

FIG. 10. *Effect of parotid fluid on glucose uptake by S. mitis 9811. Parotid fluid was present at a concentration of 50% (vol/vol).*

In contrast to the experiment shown in Fig. 4, in which no supplements were included, we supplemented saliva dilutions with either H_2O_2 alone or H_2O_2 plus SCN^-. Supplementation with H_2O_2 plus SCN^- is taken as a condition which will allow us to estimate the concentration of lactoperoxidase. Supplementation with H_2O_2 is presumed to allow the estimation of the concentration of SCN^-. The results of these experiments are shown in Fig. 11. When H_2O_2 and SCN^- were added, saliva had to be diluted ca. 1:100 before inhibition of glucose uptake was reduced. Complete loss of inhibition occurred at a saliva dilution of ca. 1:500. When H_2O_2 alone was added, complete loss of inhibition was observed at a saliva dilution of ca. 1:10. When diluted saliva alone (i.e., no H_2O_2 or SCN^- added) was tested, a complete loss of inhibition of glucose uptake occurred at a saliva dilution of ca. 1:20 to 1:30 (Fig. 4). In this latter case, the source of H_2O_2 was the organism, and H_2O_2 was certainly present in subsaturating levels.

It has been reported that H_2O_2 may inactivate lactoperoxidase (43). Therefore, to determine whether the loss of lactoperoxidase activity may account for the shift to the right of the H_2O_2 curve in Fig. 11 relative to the H_2O_2-plus-SCN^- curve, we incubated 10% saliva (i.e., a 1:10 dilution of saliva) with 100 μM H_2O_2 under the same conditions used in the experiment shown in Fig. 11. Lactoperoxidase activity was then estimated (Table 2). We observed that over a 10-min preincubation with H_2O_2, a 20 to 25% loss of lactoperoxidase activity occurred. Incubation of 10% saliva with 100 μM H_2O_2 for 60 min typically resulted in a loss of 60 to 80% of the lactoperoxidase activity. At a concentration of 50% saliva, 100 μM H_2O_2 caused a loss of enzyme activity of less than 10% after 60 min. Thus, inactivation of lactoperoxidase by H_2O_2 is dependent on saliva concentration, H_2O_2 concentration, and length of incubation. Therefore, although H_2O_2 may indeed inactivate lactoperoxidase in saliva, the experiments of Table 2 and those described above suggest that the data of Fig. 11 for H_2O_2 addition were not due to a major loss of lactoperoxidase activity. This interpretation is further supported by the saliva dilution experiment of Fig. 4, in which no exogeneous H_2O_2 was added. This study yielded a dose-response relationship which was similar to the H_2O_2 data of Fig. 11. Therefore, the data in Fig. 11 suggest that saliva contains a 500-fold excess of lacto-

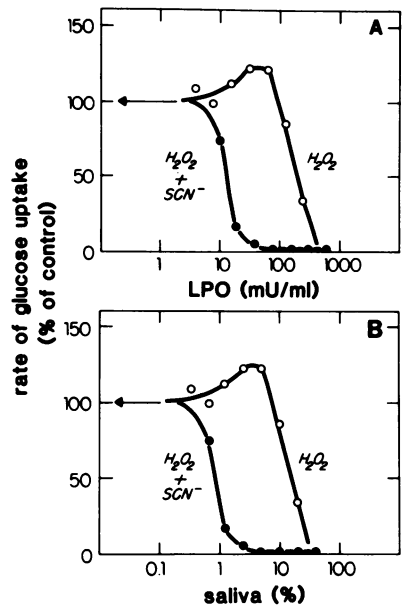

FIG. 11. *Effect of SCN^- and H_2O_2 on saliva inhibition of glucose uptake by S. mutans BHT. H_2O_2 was present at a concentration of 100 μM, and SCN^- was present at a concentration of 1 mM. Data are displayed as functions of lactoperoxidase (LPO) activity (A) and saliva concentration (B).*

TABLE 2. *Effect of H_2O_2 on the stability of salivary lactoperoxidase*

Preincubation conditions[a]	Lactoperoxidase activity[b]			
	Expt A		Expt B	
	U/ml	%	U/ml	%
No addition	1.67	100	1.40	100
H_2O_2 (100 μM)	1.24	74	1.10	79

[a] Preincubation was for 10 min at 37°C.

[b] The salivary supernatant (10%, vol/vol) served as the source of lactoperoxidase.

peroxidase and a 10-fold excess of SCN^-. Excess is used here to denote the relative concentration present in undiluted saliva as compared with the minimal amount required to give inhibition of glucose uptake (estimated as the reciprocal of the saliva dilution at which inhibition is lost).

DISCUSSION

Most studies have found that the major bacterial growth-inhibiting activity of saliva is dependent upon a non-dialyzable, heat-labile component plus a dialyzable, heat-stable component (8, 15, 41). In addition, salivary inhibition of growth of catalase-positive organisms, but not catalase-negative organisms, requires supplementation with H_2O_2 (15, 34). The dialyzable heat-stable component has been identified as SCN^-, and peroxidase activity has been associated with the non-dialyzable heat-labile component. Subsequent studies in which purified lactoperoxidase, SCN^-, and H_2O_2 were used demonstrated that this system could inhibit bacterial growth, carbohydrate fermentation, and transport of carbohydrate (15, 27, 29, 33, 34, 43–46).

The mechanism of action of the salivary lactoperoxidase-SCN^--H_2O_2 system is emerging. Recent studies identified the antibacterial component as hypothiocyanite ion ($OSCN^-$) (17). The formation of $OSCN^-$ results from the lactoperoxidase-mediated oxidation of SCN^- and the reduction of H_2O_2. $OSCN^-$ is able to oxidize protein sulfhydryls to yield sulfenyl thiocyanate derivatives, which may spontaneously give rise to either protein disulfides or protein sulfenic acids (3). It has also been demonstrated that SCN^- adducts may be formed with aromatic amino acid residues of proteins (4). Thiocyanate is regenerated from $OSCN^-$ upon oxidation of protein sulfhydryl groups (3). Thiol-reducing agents, such as cysteine, DTT, and glutathione, destroy $OSCN^-$ and may also regenerate the protein sulfhydryl groups which had been oxidized by $OSCN^-$ (46). These reducing agents also reverse the biological effects of the reconstituted lactoperoxidase-SCN^--H_2O_2 system (18, 27, 33, 34, 46).

We observed that the presence of saliva affected glucose uptake by all of the bacteria examined. All of the catalase-positive bacteria (*A. viscosus*, *S. aureus*, *E. coli*) exhibited enhanced glucose uptake in the presence of saliva. With the exception of *S. mitis*, the catalase-negative strains (*S. mutans*, *S. sanguis*) exhibited reduced glucose uptake in the presence of saliva. Saliva inhibition of glucose uptake was dependent upon a heat-sensitive factor and a dialyzable factor and was promoted by exogenous H_2O_2. The addition of H_2O_2 to saliva-suspended catalase-positive bacteria resulted in an inhibition of glucose uptake. Furthermore, DTT prevented salivary inhibition of glucose uptake. Taken together, these observations suggest that the lactoperoxidase system in saliva is responsible for the inhibition of glucose uptake observed here.

The mechanism of saliva inhibition of glucose uptake most likely depends on the oxidation of sulfhydryls on the membrane-bound transport proteins of the glucose phosphoenolpyruvate-dependent phosphotransferase transport system. The oral streptococci utilize the phosphotransferase system to transport a variety of sugars (36, 37). It has also been reported that thiol reagents inhibit phosphotransferase-mediated sugar transport in *E. coli* and that membrane-associated enzyme complex II is the target of such reagents (21). Recently, the lactoperoxidase-SCN^--H_2O_2 system was also shown to inhibit phosphotransferase-mediated glucose transport in *Streptococcus agalactiae* (27). Sugar uptake was restored by DTT.

We have no explanation for the saliva enhancement of glucose uptake and can only note that enhancement of growth and valine incorporation by *E. coli* in saliva have been reported (15). One might argue that all microorganisms actually exhibit enhanced (compared with our buffer controls) glucose uptake in saliva. Failure to observe enhancement with *S. mutans* and *S. sanguis* might be due to our inability to abolish completely the action of the lactoperoxidase system by heating or including DTT in our experiments. Alternatively, other unknown factors in saliva might differentially affect *S. mutans* and *S. sanguis* strains. We have occasionally observed that at high dilutions of saliva the rate of glucose uptake by *S. mutans* BHT exceeds by 20 to 100% the rate of uptake in buffer-suspended controls (Fig. 11) (unpublished data). Thus, it appears possible that some salivary factor(s) might be able to limit glucose uptake in *S. mutans* and *S. sanguis*, but not in the catalase-positive organisms or *S. mitis*, such that rates that exceed the rates in buffer-suspended control cells are observed only upon dilution of the saliva specimens. The relationship (if any) between the saliva-induced enhancement of glucose uptake reported here and the salivary enhancement of glycolysis by salivary sediment or bacterial cultures reported previously is unknown (19). Further studies will be necessary to understand the basis and significance of the saliva-induced enhancement phenomenon.

S. mitis 9811 was consistently refractory to saliva inhibition of glucose uptake. In fact, saliva always stimulated glucose uptake compared

with buffer-suspended cells in this catalase-negative microorganism. Experiments with another strain of *S. mitis* (72 × 41) yielded the same results as those reported here for strain 9811. Exogenous H_2O_2 promoted the inhibition of glucose uptake by saliva-suspended cells. Furthermore, *S. mitis* exhibited a sensitivity to added H_2O_2 which was similar to that of *S. mutans*. Thus, it appears that *S. mitis* failed to accumulate sufficient H_2O_2 under the conditions of our experiments to promote inhibition of glucose uptake. Production of H_2O_2 by oral *S. mitis* has been observed previously (16, 20). Experiments in progress suggest that H_2O_2 production by *S. mutans* BHT and by *S. mitis* 9811 is essentially identical under a variety of conditions in the absence of saliva. It is not known whether the production of H_2O_2 by *S. mitis* is particularly sensitive to saliva compared with H_2O_2 production by *S. mutans*. It is possible that certain substrates in saliva might be more readily available as sources of reducing power to *S. mutans* than to *S. mitis*. It is also possible that *S. mitis* may accumulate less H_2O_2 in saliva as a result of altered activity of reduced nicotinamide adenine dinucleotide oxidase or reduced nicotinamide adenine dinucleotide peroxidase. Accumulation of H_2O_2 by group N streptococci has been related to the levels of these two enzyme activities (1). It has been reported that there are strains of group N streptococci which are resistant to the lactoperoxidase-SCN^- system even when exogenous H_2O_2 is supplied (29, 30). Resistance was related to the presence of an enzyme that oxidized reduced nicotinamide adenine dinucleotide in the presence of an oxidation product of SCN^-. Since *S. mitis* displayed sensitivity to saliva in the presence of exogenous H_2O_2, the mechanism of resistance in *S. mitis* is clearly different than the mechanism of resistance in the group N streptococci described above. Further studies of H_2O_2 production by buffer- and saliva-suspended cells of *S. mitis* and *S. mutans* will be necessary to establish the basis of the difference between these two microorganisms.

The initial saliva-dependent burst of glucose uptake observed only with *S. mutans* and *S. sanguis* strains remains unexplained. Since the initial burst was potentiated by heated saliva and was abolished by H_2O_2 or NaF, it appears to be sensitive to the lactoperoxidase system and perhaps dependent on the phosphotransferase transport system.

The effective operation of the lactoperoxidase system in the oral cavity depends upon the presence of the enzyme, SCN^-, and a source of H_2O_2. The oral microflora is generally considered to be the principal source of H_2O_2. Our results (Fig. 11) suggest that lactoperoxidase is present in saliva at levels ca. 500 times larger than the level needed to inhibit glucose uptake. The concentration of bacteria used in our studies (ca. 10^9 cells per ml) approximated the total bacterial load typical of whole saliva. The effective concentration of SCN^- in saliva was estimated to be 10 to 20 times greater than the concentration required to reduce glucose uptake. Our analyses of SCN^- in the saliva specimens used in these experiments yielded values on the order of 500 to 600 μM. Thus, ca. 30 to 60 μM SCN^- would be sufficient for inhibition of glucose uptake. In light of our estimates of the effective concentrations of lactoperoxidase and SCN^-, efforts to relate the lactoperoxidase system to oral health might more properly focus on SCN^- measurements rather than on the enzyme since SCN^- is more likely to reach limiting levels than the enzyme is.

In the presence of 50% (vol/vol) saliva, about 50 μM H_2O_2 was required to maximally inhibit glucose uptake; 50% inhibition occurred at about 10 μM H_2O_2. In a previous study (20) it was suggested that H_2O_2 concentrations in saliva were less than ca. 30 μM. This estimate was based on the limit of detection of the assay technique used. Low H_2O_2 levels in saliva were thought to most likely result from the rapid degradation of H_2O_2 by salivary peroxidase and catalase. It was further suggested that due to the low H_2O_2 levels that must occur in saliva, the effect of the H_2O_2 on the oral microflora might be insignificant. Our results suggest that only low levels of H_2O_2 are required to observe the effects of the lactoperoxidase system. Furthermore, if the long-term effects of a small impairment of the metabolic potential of a given bacterium are considered, it seems clear that such affected cells would eventually become less competitive than other unaffected cells. Previous reports have suggested that the availability of H_2O_2 is the limiting component of the lactoperoxidase system in vivo (43). Our results are consistent with this hypothesis if one considers only those situations where complete inhibition of the function under study is taken as the end point. We argue that even a partial impairment of function can be significant to the microbial ecology of the mouth.

In the absence of added H_2O_2, complete inhibition of glucose uptake was never observed. In the case of the *S. mutans* strains, 30 to 80% inhibition of the rate of glucose uptake was observed. The BHT strain of *S. mutans* consistently exhibited about 50% inhibition of glucose uptake. The above-described levels of inhibition were observed in 50% (vol/vol) saliva. Increases

in the percentage of saliva did not increase the degree of inhibition. Production of H_2O_2 in the oral cavity is dependent upon the availability of O_2 and appropriate bacteria. A recent study reported that parotid fluid and whole saliva were 25 to 75% saturated with respect to O_2 (13). Thus, O_2 is probably not limiting in saliva. However, in plaque the O_2 concentration decreases with depth (i.e., as the tooth surface is approached) (13). In nonplaque locations, bacterial production of H_2O_2 or H_2O_2 stability (rather than an insufficient supply of O_2) may preclude accumulation to nonlimiting levels. In the depths of plaque O_2 availability (rather than bacteria or lactoperoxidase [32, 45]) is most likely limiting. Furthermore, since both lactoperoxidase and catalase are present in the mouth, intraoral H_2O_2 metabolism is probably partitioned among at least two enzymatic systems. Catalase might be expected to diminish H_2O_2 availability for lactoperoxidase-mediated oxidation of SCN^- (20, 28).

Finally, in the oral cavity the impact of the salivary lactoperoxidase system may be distributed unevenly among diverse sites. The greatest impact might be expected in those locations that are continuously bathed by renewed salivary secretions. The least impact would be expected in plaque accumulations. In this regard, one might expect (strictly on the basis of the lactoperoxidase system) that those microorganisms dependent upon a nonshedding surface (i.e., a surface that is retained long enough to allow plaque to accumulate) for continued presence in the oral cavity might fail to survive on shedding surfaces because of their susceptibility to the lactoperoxidase system. Accordingly, those microorganisms that colonize locations continuously exposed to fresh saliva might be relatively resistant to the lactoperoxidase system. Furthermore, if these latter organisms could also survive in plaque, they would be distributed universally around the various oral sites. In our studies *S. mutans* and *S. sanguis* were susceptible to the inhibitory action of saliva alone. These microorganisms require a nonshedding surface in order to maintain a continuous presence in the mouth (12). *S. mitis* and *A. viscosus* were refractory to the inhibitory action of saliva alone. These microorganisms are not dependent on a nonshedding surface for continued presence and are more or less universally distributed (especially *S. mitis*) around the oral cavity (12). Thus, it is possible that the intraoral distribution of these four species is dependent to a large extent on their in situ susceptibilities to the salivary lactoperoxidase system. Further study of these and additional species may allow a reasonable assessment of the impact of the salivary lactoperoxidase system on the site preferences exhibited by members of the oral microflora.

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