Bacteriolytic Action of Fluoride Ions

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Bacillus subtilis, *Neisseria subflava*, and LYT coccus were found to undergo massive lysis after growth in media containing 0.01 to 10 mM NaF. When cells of these organisms were transferred from late-exponential-phase cultures to 0.02 M sodium phosphate buffer plus 0.1 M KCl, they underwent spontaneous autolysis. Cells grown in media with fluoride were more liable to autolysis, and walls isolated from them also showed enhanced autolytic sensitivity, even though added fluoride did not directly stimulate autolysins. Sporadic or partial lysis occurred in populations of *Streptococcus sanguis* and *Streptococcus mutans* BHT or LM-7 after growth in fluoridated media. Most bacteria that were tested did not undergo fluoride-induced lysis. However, cells of all test bacteria were found to have reduced amounts of peptidoglycan per unit of cell weight when grown in the presence of fluoride. Incorporation of labeled lysine or glucosamine into peptidoglycan (Park-Hancock residue) was stimulated, instead of inhibited, by fluoride. However, fluoride also stimulated the loss of radioactivity from Park-Hancock residues of cells that had previously incorporated labeled lysine or glucosamine. Thus, fluoride appeared to enhance peptidoglycan turnover, and this turnover reduced the peptidoglycan contents of all bacteria tested, but induced lysis in only those bacteria that normally have highly active autolytic systems.

There is currently much interest in the physiological actions of fluoride ions on bacterial cells, primarily because fluorides are effective anticaries agents, and it is felt that at least part of their effectiveness may be due to the direct inhibition of the metabolism of plaque bacteria (7). For example, enolase (EC 4.2.1.11) is relatively susceptible to the inhibitory action of fluoride, and Kanapka and Hamilton (6) presented evidence to indicate that 2.4 mM NaF can inhibit the enzyme in intact, growing cells of *Streptococcus salivarius*. They found that there was a decline in intracellular levels of phosphoenolpyruvate and pyruvate in fluoride-treated cells and a rise in 2-phosphoglycerate. The decline in phosphoenolpyruvate was associated with the reduced uptake of glucose by the phosphotransferase system and an overall slowing of glycolysis. Fluoride ions are known to interfere with a number of other processes in bacteria, for example, cation translocation (9), polysaccharide storage (5), and extracellular polysaccharide synthesis (1); in fact, fluoride is inhibitory for many enzymes besides enolase (18), and therefore a multiplicity of physiological effects would be expected.

Since fluoride can reduce phosphoenolpyruvate supply in intact cells, we felt that it might also inhibit peptidoglycan synthesis, specifically in the formation of uridine 5'-diphos-

phate (UDP)-*N*-acetylglucosamine-3-enol-pyruvyl ether from UDP-*N*-acetylglucosamine and phosphoenolpyruvate. In experiments designed to test this hypothesis, we were surprised to find that some bacteria responded to fluoride in growth media by undergoing massive lysis. Our initial characterization of the lytic phenomenon is described in this paper.

MATERIALS AND METHODS

Bacteria and growth conditions. *Bacillus subtilis* BR151 was obtained from G. Wilson of the University of Rochester. Strains AHT, BHT, LM-7, and SL-1 of *Streptococcus mutans* were obtained from R. Herbison of the Eastman Dental Center in Rochester, N.Y.; strain GS-5 was obtained from G. D. Shockman of Temple University. *Bacterionema matruchotti* was obtained from M. Gilmour of the Eastman Dental Center. *Neisseria subflava* was from our own culture collection, and all other bacteria used were standard strains.

Lytic (LYT) coccus was isolated during this investigation as a contaminant in a culture of *S. mutans*. To date, we have not identified it in terms of genus and species. It is particularly useful because it has a highly active autolytic system and undergoes fluoride-induced bacteriolysis. It occurs as single cells, pairs, or short chains. It is facultatively anaerobic, gram positive, oxidase negative, strongly catalase positive, and very weakly hemolytic on sheep blood agar. It produces acid but no gas

from sucrose, mannitol, glucose, trehalose, and xylose, but not from sorbitol, raffinose, lactose, galactose, or ribose.

All bacteria were routinely grown at 37°C in the tryptone-glucose-Marmite medium (TGM) described previously (11), except for *N. subflava* which was grown with gentle shaking in a medium containing 2.5% (wt/vol) brain heart infusion (Difco), 0.3% yeast extract (Difco), and 2% glucose. The absorbance or turbidity of cultures was assessed by use of a Beckman model DU spectrophotometer set for light of 700-nm wavelength and with 1-cm light path cuvettes.

For some experiments, bacteria were grown in pellet cultures, as described previously (10). For these cultures, tubes of medium are inoculated and immediately centrifuged to pellet the inoculum bacteria, which were then allowed to grow in the pellets with intermittent centrifugation. For sampling, cells were resuspended by use of a Vortex mixer; a sample was withdrawn, and the culture was immediately centrifuged.

Cell fractionation. Cell walls were isolated by standard techniques, which involved the breakage of cells with sonic treatment and then differential centrifugation. The purity of wall preparations was assessed by phase microscopy, by light-scattering analysis, and by the determination of the reactivity of the preparations with the Folin reagent (3).

Peptidoglycans were isolated from whole cells or from isolated walls by means of the Park-Hancock fractionation technique (13), which involves initial cold 5% trichloroacetic acid treatment, followed by ethanol extraction, hot (90°C for 6 min) trichloroacetic acid extraction, and trypsin digestion. The fractionation was designed to yield a residue of pure peptidoglycan. Accessory wall polymers such as teichoic acids or proteins are usually removed during fractionation. The residues we obtained were devoid of proteins or nucleic acids as indicated by absorption-scattering curves and by essentially nil reactivity with the Folin (8) and orcinol (3) reagents. Acid-hydrolyzed residues yielded less than 0.02% (by weight) inorganic phosphate, and, therefore, teichoic acids must have been lost during fractionation. The residues could be solubilized with lysozyme.

Labeling experiments. Peptidoglycans could be radioactively labeled with D-[1-¹⁴C]glucosamine (ICN Inc., Irvine, Calif.) added to growth media to yield a level of 0.05 μ Ci/ml. The specific activity of the added compound was 58.2 mCi/mmol. Media used for labeling were: brain heart infusion for *N. subflava*, the defined, minimal medium of White et al. (17) for *S. mutans*, and Spizizen (16) minimal medium for *B. subtilis*. When tritiated lysine (ICN, Inc.; 8 Ci/mmol) was used to label the peptidoglycan of cells, it was incorporated into the growth medium at an activity of 0.1 μ Ci/ml. Five percent inocula from stationary-phase cultures were used, and the cells were allowed to grow for 24 h before harvest to allow for maximal label incorporation. After harvest, the cells were washed twice with deionized water and suspended in water. The total radioactivity was determined by pipetting 0.1 ml of suspension

into 10 ml of Aquasol scintillation fluid (New England Nuclear, Boston, Mass.) and then placing the mixtures in a Beckman model LS-233 counter. Part of the initial cell suspension was dried in a vacuum oven to constant weight for an estimate of cell dry weight.

The fraction of the total incorporated label that was in peptidoglycan was estimated by fractionating whole cells by the Park-Hancock procedure and then comparing the radioactivity of the residue with that of whole cells.

For turnover experiments, washed labeled cells were used to inoculate fresh media. Generally, about 5 mg (wet weight) of cells was used to inoculate 100 ml of medium. The cells were allowed to grow for up to 24 h at 37°C, harvested, washed, and suspended in water, and their radioactivity was assayed. The cells were then fractionated by means of the Park-Hancock procedure to assess the loss of label from peptidoglycan. Mass doublings for the cultures were estimated from plots of optical density versus time.

RESULTS

The growth curves shown in Fig. 1 for *B. subtilis* BR151 in media with 0, 1, 5, or 10 mM NaF are similar to ones obtained for *N. subflava* or LYT coccus. They show that 1 mM NaF had little effect on growth, whereas 5 or 10 mM fluoride significantly reduced both the rate of growth and the culture yield, expressed here in terms of absorbance. However, the most dramatic effect shown in Fig. 1 is the massive lysis that occurred after growth had ceased in the presence of 5 or 10 mM NaF. A similar, extensive lytic response occurred in cultures of *N. subflava* or LYT coccus after growth in the presence of 5 or 10 mM NaF. Microscopic examination of the cultures that had undergone major decreases in absorbance revealed very few intact cells and much cell debris.

It is possible to grow *B. subtilis*, *N. subflava*, or LYT coccus in pellet cultures at the bottom of centrifuge tubes. The bacteria grow less rapidly in the crowded conditions of pellets than in ordinary suspension cultures, but, still, they are able to survive and multiply in this situation that mimics the natural crowding of many ecological niches in soil or on tooth surfaces. Fluoride-induced lysis occurred in pellet cultures; data for *B. subtilis* is shown in Fig. 1.

Experiments with a variety of bacteria indicated that the lytic responses of *B. subtilis*, *N. subflava*, and LYT coccus were extreme ones. *Streptococcus sanguis* consistently exhibited a lytic response after growth in media with 0.5 to 5 mM NaF, but only about one half the population lysed as indicated by direct counts. *S. mutans* strain BHT or LM-7 did undergo fluoride-induced lysis, but the response was sporadic and occurred in only some of the cultures

FIG. 1. Growth and lysis of *B. subtilis* BR151 at 37°C. Data are shown for pellet and suspension cultures in TGM medium plus 0 mM NaF (○), 1 mM NaF (△), 5 mM NaF (□), and 10 mM NaF (▽). Suspension cultures were grown statically in test tubes. A₇₀₀, Absorbance at 700 nm.

tested. In addition, *Streptococcus pneumoniae* and *Staphylococcus aureus* H underwent lysis more readily after growth in media with 5 or 10 mM NaF than after growth in unsupplemented media. The other bacteria we tested did not undergo lysis in the presence of fluoride. In addition to the organisms listed in Table 2, they included: *Escherichia coli* strain B, *Actinomyces viscosus* ATCC 15987, *Streptococcus faecalis* ATCC 9790, *Lactobacillus plantarum* ATCC 8014, and *Neisseria flavescens* ATCC 13120.

It is a general finding that bacteria are more fluoride susceptible at low pH (7). Acid conditions also favored the lytic response, and it was possible to obtain massive lysis of LYT coccus cultures with only 0.01 mM NaF if the initial culture pH was decreased from 7 to 6. Further pH reduction to 5.5 or 5.0 resulted in so little growth with even 0.01 mM NaF that it was difficult to assess lysis. Control cultures were able to grow at these low pH values and to reduce the pH to about 4.6. In effect, fluoride seemed to render the cells more acid susceptible. Growth in the presence of fluoride resulted in less acidification of the cultures. For example, final pK values for static LYT coccus cultures were about 6.2, 5.6, 5.0, and 4.6 in the presence of 10, 5, 1, and 0 mM NaF. Final lactate concentrations were 10.6, 12.7, 15.1, and 19.4 mM, respectively. Thus, the pH in cultures in which massive lysis occurred was sufficiently high so there would be little HF formed (the pH of HF is 3.2), and we concluded that the active form of fluoride is the anion F⁻.

B. subtilis and LYT coccus were less susceptible to fluoride when grown with agitation-aeration on a rotary shaker, and fluoride concentrations as high as 50 mM were required to reduce the growth yield by approximately 50%. The lytic response also was diminished in aerated cultures; for example, even after 120 h of incubation, the optical densities of LYT coccus cultures with 20 mM NaF were still nearly 50% of the maximal value. *B. subtilis* cultures showed less lysis, and final optical densities were about 90% of the maximal values for aerated cultures with 50 mM NaF. The higher pH of aerated cultures seemed important here, and it was possible to increase fluoride resistance in static or pellet cultures of *B. subtilis* or LYT coccus simply by maintaining the pH above 7 with intermittent base addition.

N. subflava behaved differently under agitation-aeration conditions. Growth could be significantly inhibited by 5 or 10 mM NaF. The mass doubling time of 1.7 h in medium without fluoride was increased to 2.4 h in the presence of 5 mM NaF and 4.2 h with 10 mM NaF. Maximum optical densities were reduced by 41 and 85%, respectively, and massive lysis followed shortly after maximum growth was attained.

Autolysis and fluoride-induced lysis. The particular organisms that proved to be susceptible to fluoride-induced lysis suggested to us that autolysins might be involved in the phenomenon. Certainly, *B. subtilis* BR151 is known to have a highly active autolytic system, and Fig. 2 shows that cells of LYT coccus re-

moved from cultures in the late exponential phase underwent spontaneous lysis when transferred to 0.02 M sodium phosphate buffer with 0.1 M KCl. Lysis was most extensive at pH 6, and cells grown in the presence of 10 mM NaF were significantly more liable to lysis at pH 5 or 6 than were cells grown in fluoride-free medium. The addition of fluoride to the buffered suspensions had no effect on lysis. *N. subflava* also had an active autolytic system, and washed cells previously grown in the presence of 10 mM NaF underwent 61% lysis during 10 h of incubation in 0.02 M sodium phosphate

FIG. 2. Autolysis of whole cells of LYT coccus in 0.02 M sodium phosphate buffer plus 0.1 M KCl solution adjusted to various pH values. Data are shown for cells grown in TGM medium (○) or TGM medium plus 10 mM NaF (△), as well as for heat-inactivated cells (□). The temperature for lysis was 37°C, and the time was 24 h. A_{700} , Absorbance at 700 nm.

TABLE 1. Autolysis of cell walls isolated from LYT coccus

Walls ^a isolated from cells grown in TGM plus:	Incubation time ^b (h)	% Reduction in A_{700} ^c at pH:		
		5.0	6.0	7.0
0 mM NaF	2	12	16	8
	4	19	21	11
	24	28	48	24
10 mM NaF	2	16	23	14
	4	26	27	21
	24	60	72	32

^a Walls were isolated from cells from cultures in the late exponential phase of growth.

^b Incubations were at 37°C in 0.02 M sodium phosphate buffer plus 0.1 M KCl.

^c A_{700} , Absorbance at 700 nm.

buffer (pH 6.0) plus 0.1 M KCl. Cells from fluoride-free cultures underwent only 40% lysis.

Walls were isolated from LYT coccus by means of differential centrifugation of cells disrupted by sonic treatment. Table 1 shows that walls isolated from cells grown in medium with 10 mM NaF underwent more extensive autolysis than did walls isolated from cells grown in fluoride-free medium. As with whole cells, pH 6 was optimal for lysis. Also, the best suspending medium for lysis was 0.02 M sodium phosphate buffer with 0.1 M KCl. Other media tested included 0.02 M sodium phosphate buffer without KCl and 0.02 M potassium phosphate buffer with or without 0.1 M KCl.

The lytic reaction in cultures could be prevented by adding salt to growth media. For example, 80 mM CaCl_2 or MgCl_2 completely suppressed the lytic response; 50% inhibition was obtained with concentrations of about 70 mM. Sodium chloride and potassium chloride were effective also, although somewhat higher concentrations of about 150 mM were required for the complete suppression of the lytic effect.

Bacteriolysis of LYT coccus, *B. subtilis*, and *N. subflava* could also be induced with penicillin. In the example shown in Fig. 3, the effects of 0.01 to 0.10 μg of penicillin G per ml on LYT coccus can be compared with the effects of 5 or 10 mM NaF. Both agents cause lysis after growth. With penicillin, growth inhibitory and lytic effects were more closely associated, and it was difficult to obtain nearly full growth followed by nearly complete lysis. Presumably, the specific biochemical targets of the two agents differ, and penicillin G was about 10,000 times more potent than F^- on a molar basis. However, both are lytic and bactericidal. Penicillin-induced lysis occurred somewhat more rapidly than did fluoride-induced lysis; both occurred optimally at a pH of approximately 6.

Peptidoglycan content of fluoride-treated cells. Enhanced mureolytic activity or direct inhibition of peptidoglycan synthesis by fluoride action should result in cells that are deficient in peptidoglycan content. Table 2 indicates that cells of a variety of bacteria have less peptidoglycan (Park-Hancock residue) per unit of cell weight when grown in media supplemented with 5 mM NaF than when grown in unsupplemented media. This reduction is apparent even with organisms that do not undergo lysis. In view of the rather substantial reductions in peptidoglycan contents of bacteria grown in the presence of fluoride, we were surprised to find that fluoride had no inhibitory effect, but a stimulatory effect, on the incorporation of labeled precursors into Park-Hancock residues.

FIG. 3. Lysis of LYT coccus induced by NaF and penicillin G. Cultures were grown statically at 37°C in TGM medium. A_{700} , Absorbance at 700 nm.

TABLE 2. Effect of NaF on peptidoglycan contents of bacteria

Organism	Peptidoglycan content (% dry weight) of cells grown in TGM plus:	
	0 mM NaF	5 mM NaF
<i>B. subtilis</i>	22.1	15.2
LYT coccus	12.7	5.4
<i>S. sanguis</i> 10556	12.6	6.8
<i>S. salivarius</i> 13419	13.0	7.9
<i>S. mutans</i> AHT	16.0	11.0
<i>S. mutans</i> BHT	21.5	15.0
<i>S. mutans</i> GS-5	19.9	13.0
<i>S. mutans</i> SL-1	24.0	16.0
<i>S. mutans</i> LM-7	18.0	12.0
<i>Rothia dentocariosa</i> 17931	18.4	13.0
<i>B. matruchotii</i>	18.0	11.2

Typical data are shown in Table 3, where it is apparent that the incorporation of tritiated lysine into Park-Hancock residues of growing cells of *N. subflava* or *S. mutans* was stimulated by 5 mM NaF. Similar results were obtained when glucosamine was used for labeling.

Loss of label from Park-Hancock residues. Since fluoride enhances autolytic activity, it seemed that it might also increase the turnover

of cell wall polymers that would be reflected in the loss of label from cells that had previously incorporated radioactive substrates into wall polymers. Table 4 shows that fluoride does increase the turnover or loss of label from Park-Hancock residues of cells that had been grown in the presence of [14 C]glucosamine. These losses, which were apparent also when whole cells instead of residues were assayed, are presumably due to the enhanced turnover of peptidoglycan.

S. mutans has been found by Shockman et al. (15) to conserve its peptidoglycan and, as Table 4 shows, the SL-1 and LM-7 strains lose very little label from Park-Hancock residues during growth—only about 3% per mass doubling. However, in the presence of 5 mM NaF, the SL-1 strain showed a dramatic increase in turnover to about 56% per mass doubling, and the LM-7 strain showed a less dramatic but marked increase due to fluoride. *N. subflava* and *B. subtilis*, both of which have highly active autolytic systems, showed extensive turnover even in the absence of fluoride, but this turnover was enhanced by fluoride. With these latter two bacteria, care was taken to harvest cultures before the lytic phase so lysis would not contribute to the measured loss of label. Moreover, cells labeled with tritiated thymidine showed

TABLE 3. Effect of NaF on L-[³H]lysine incorporation into Park-Hancock residues

Organism	Fluoride concn in growth medium (mM)	Time after addition of [³ H]lysine (h)	<i>A</i> ₇₀₀ ^a	Radioactivity in Park-Hancock residue	
				cpm	cpm/mg
<i>N. subflava</i>	0	0 ^b	0.43		
		1	0.53	1,046	301
		2	0.64	1,564	370
		3	0.70	1,951	434
	5	0 ^b	0.37		
		1	0.43	799	322
		2	0.50	2,309	775
		3	0.57	2,487	734
<i>S. mutans</i> SL-1	0	0 ^c	0.41		
		1	0.46	2,108	313
		2.5	0.71	3,358	334
		4	1.15	5,844	348
	5	0 ^c	0.18		
		1	0.20	1,294	365
		2.5	0.22	1,693	455
		4	0.24	1,883	444

^a *A*₇₀₀, Absorbance at 700 nm.^b Cultures were grown at 37°C in brain heart infusion broth for 4 h prior to the addition of [³H]lysine to yield 10 μCi/100 ml.^c Cultures were grown at 37°C in the defined medium of White et al. (17) for 10 h prior to the addition of [³H]lysine.

TABLE 4. Fluoride-enhanced loss during growth of previously incorporated, labeled glucosamine from Park-Hancock residues

Organism	Fluoride concn in growth medium (mM)	<i>A</i> ₇₀₀ ^a		Radioactivity (cpm) in Park-Hancock residue		% Loss	% Loss per mass doubling
		Initial	Final	Initial	Final		
<i>S. mutans</i> SL-1	0	0.17	1.22	1,489	1,380	7	3
	5	0.16	0.42	1,489	411	72	56
<i>S. mutans</i> LM-7	0	0.16	1.20	2,395	2,250	6	3
	5	0.16	0.54	2,395	1,752	27	16
<i>N. subflava</i>	0	0.20	0.95	2,120	870	59	27
	5	0.20	0.72	2,120	719	66	36
<i>B. subtilis</i> BR151	0	0.09	0.24	2,025	920	55	42
	5	0.09	0.18	2,025	811	60	60

^a *A*₇₀₀, Absorbance at 700 nm.

at most a 5% (in most cases less than 1%) loss of label per mass doubling. Thus, it appeared that the loss of label from the Park-Hancock residues was due to a selective loss of peptidoglycan rather than to cell lysis in the population during growth.

DISCUSSION

A major finding presented here is that fluoride ions can be bactericidal for certain bacteria in that they induce a lytic response. Moreover, it appears that the noncidal inhibition of bacte-

rial growth by fluoride also involves the derangement of peptidoglycan retention in the cell wall. We had initially thought that fluoride inhibited peptidoglycan synthesis by reducing the supply of phosphoenolpyruvate used in the initial stages of synthesis. However, we obtained no evidence for such inhibition from determinations of the effects of fluoride on the incorporation of labeled glucosamine or lysine into Park-Hancock residues. At present, it appears that fluoride causes lysis primarily by enhancing the autolytic activity of cells in cul-

ture. Fluoride ions do not enhance the autolysis of cells grown in media without fluoride and then suspended in buffer. Thus, fluoride does not appear to affect the activity of autolysins directly, but causes some change during growth that leads to enhanced activity. This change could involve a greater production of autolytic enzymes or substrate changes that lead to greater susceptibility of autolysins. Presumably, the inhibition of fluoride-induced lysis by salts is due to the displacement of autolysins from the wall by the salts (14), but other electrophysiological effects may be involved.

The role of the responses described here in the anticaries activity of fluoride is an open question. Fluoride levels in saliva tend to be rather low, about 0.005 mM, even in areas with fluoridated water supplies. However, fluoride is concentrated by dental plaque. Dawes et al. (4) measured average fluoride levels of 47 ppm, or 2.5 $\mu\text{mol/g}$ (wet weight), for plaque samples from people living in areas with fluoridation of the water supply. Individual plaque samples were found to contain as much as 170 ppm, or 8.9 $\mu\text{mol/g}$. Recently, a dramatic success in caries reduction has been achieved with topically applied fluorides, especially in xerostomia patients who have reduced saliva production and normally have rampant caries (2). Acidulated fluoride gels or fluoride mouth washes commonly contain as much as 1 M fluoride, and, therefore, plaque bacteria are exposed to very high levels of fluoride for relatively short periods of time. Longer-acting, slow-release fluoride preparations are currently being developed (12), and their use should result in prolonged high levels of fluoride in plaque. In all, it seems that the inhibitory effects of fluoride for plaque bacteria involve the autolytic loss of peptidoglycan, and this loss may well render the bacteria more susceptible to other inhibitory agents, either natural ones in saliva or therapeutically imposed ones. Perhaps the most effective anticaries regimen will involve fluoride combined with some other agents that affected bacterial surface structures.

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LITERATURE CITED

1. Bowen, W. H., and M. J. Hewitt. 1974. Effect of fluoride on extracellular polysaccharide production by *Streptococcus mutans*. J. Dent. Res. 53:627-629.
2. Brown, L. R., S. Dreizen, and S. Handler. 1976. Effects of selected caries preventive regimens on microbial changes following irradiation-induced xerostomia in cancer patients, p. 275-290. In H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: microbial aspects of dental caries, special supplement to Microbiological Abstracts, vol. 3, Information Retrieval, Inc., Washington, D.C.
3. Ceriotti, G. 1955. Determination of nucleic acids in animal tissues. J. Biol. Chem. 214:59-70.
4. Dawes, C., G. N. Jenkins, J. L. Hardwick, and S. A. Leach. 1965. The relation between the fluoride concentrations in the dental plaque and in drinking water. Br. Dent. J. 119:164-167.
5. Hamilton, I. R. 1969. Studies with fluoride-sensitive and fluoride-resistant strains of *Streptococcus salivarius*. I. Inhibition of both intracellular polyglucose synthesis and degradation by fluoride. Can. J. Microbiol. 15:1013-1019.
6. Kanapka, J. A., and I. R. Hamilton. 1971. Fluoride inhibition of enolase activity *in vivo* and its relationship to the inhibition of glucose-6-phosphate formation in *Streptococcus salivarius*. Arch. Biochem. Biophys. 146:167-174.
7. Levine, R. S. 1976. The action of fluoride in caries prevention. Br. Dent. J. 140:9-14.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
9. Luoma, H., and H. Tuompo. 1975. The relationship between sugar metabolism and potassium translocation by the caries-inducing streptococci and the inhibitory role of fluoride. Arch. Oral Biol. 20:749-755.
10. Marquis, R. E., R. J. Leshner, and R. Bronsteen. 1976. Growth of plaque bacteria in pellet culture and the lytic effect of fluoride, p. 821-827. In H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: microbial aspects of dental caries, special supplement to Microbiological Abstracts, vol. 3, Information Retrieval, Inc., Washington, D.C.
11. Marquis, R. E., N. Porterfield, and P. Matsumura. 1973. Acid-base titration of streptococci and the physical states of intracellular ions. J. Bacteriol. 114:491-498.
12. Mirth, D. B., and W. H. Bowen. 1976. Chemotherapy: antimicrobials and methods of delivery, p. 249-262. In H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: microbial aspects of dental caries, special supplement to Microbiological Abstracts, vol. 1, Information Retrieval, Inc., Washington, D.C.
13. Park, J. T., and R. Hancock. 1960. A fractionation procedure for studies of the synthesis of cell-wall mucopeptide and of other polymers in cells of *Staphylococcus aureus*. J. Gen. Microbiol. 22:249-258.
14. Pooley, H. M., J. M. Porres-Juan, and G. D. Shockman. 1970. Dissociation of an autolytic enzyme-cell wall complex by treatment with unusually high concentrations of salt. Biochem. Biophys. Res. Commun. 38:1134-1140.
15. Shockman, G. D., H. C. Tsien, R. E. Kessler, M. Mychajlonka, M. L. Higgins, and L. Danco-Moore. 1976. Effect of environmental factors on the surface properties of oral microorganisms, p. 631-647. In H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: microbial aspects of dental caries, special supplement to Microbiological Abstracts, vol. 3, Information Retrieval, Inc., Washington, D.C.
16. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxynucleate. Proc. Natl. Acad. Sci. U.S.A. 44:1071-1078.
17. White, G. E., C. L. Cooney, A. J. Sinskey, and S. A. Miller. 1974. A defined continuous culture medium for *Streptococcus mutans*. J. Dent. Res. 53:762.
18. Wiseman, A. 1970. Effect of inorganic fluoride on enzymes, p. 48-97. In F. A. Smith (ed.), Pharmacology of fluorides, handbook of experimental pharmacology, vol. XX/2. Springer-Verlag, Berlin.