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Response of Freshly Isolated Strains of *Streptococcus mutans* and *Streptococcus mitior* to Change in pH in the Presence and Absence of Fluoride During Growth in Continuous Culture

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A study was undertaken to compare the effects of pH and fluoride on the growth and metabolic properties of *Streptococcus mutans* 2452 and *Streptococcus mitior* 572, strains recently isolated from 8-year-old school children and grown in continuous culture with a glucose limitation. Each experiment had four consecutive stages of growth: (i) pH 7.0, (ii) no pH control, (iii) pH 7.0, and (iv) no pH control plus 50 μg of fluoride per ml in the medium. At a dilution rate (D) of 0.13 h^{-1}, cells of *S. mitior* possessed high glycolytic activity at pH 7.0 in the initial stage, but were washing out of the chemostat within 24 h after the pH control was shut off and the pH fell to 5.1. Once the culture was reestablished at pH 7.0, fluoride (50 μg/ml) was added to the medium and the pH control was again turned off. Whereas cell numbers fell from 24.0×10^8 to 0.9×10^8/ml within 24 h, the culture remained relatively constant during the following 6 days despite the fall in pH to 5.4. The cells from this culture also maintained an intermediate glycolytic rate of 0.44 μmol mg^{-1} min^{-1}. The cells in this latter stage developed phenotypic resistant to fluoride at concentrations up to 16 mM. Growth of *S. mitior* at $D = 0.034$ h^{-1} resulted in a slower response to environmental change such that cells were able to grow to pH values as low as 5.2 in the absence of fluoride. In contrast to *S. mitior*, *S. mutans* 2452 under the same conditions at $D = 0.13$ h^{-1} grew to higher cell numbers and higher yields and was able to maintain significant cell numbers to pH 4.8 once the pH control was shut off in the presence and absence of fluoride. *S. mutans* had 40% less glycolytic activity but was fourfold more resistant to fluoride at the start of the experiment, and cells were shown to adapt to growth at low pH and to fluoride at levels as high as 20 mM. This fluoride resistance by freshly isolated *S. mutans* 2452 was significantly higher than that of *S. mutans* DR0001 grown under identical conditions in the chemostat. *S. mutans* DR0001 is a strain which has been subcultured in vitro for several years. This study demonstrated that *S. mutans* 2452 was more aciduric than *S. mitior* 572 and, unlike the latter organism, could grow at pH values below 5.1. The addition of fluoride to the medium stabilized the *S. mitior* culture in the absence of pH control, indicating that whereas fluoride does suppress growth and glycolytic activity it also results in higher environmental pH values, which permit the survival of the less aciduric bacteria.

The complex nature of dental plaque has been known for some time (1); however, the ecological relationships of the microbial populations in plaque have been the subject of relatively few studies. This has undoubtedly led, until recently, to the view that dental caries and periodontal disease are caused by single "pathogenic" agents. More recent investigations (2, 13, 16), however, have indicated that the etiology of both diseases is multifactorial and probably requires a variable mixture of pathogenic organisms.

We have undertaken an examination of the properties of various oral bacteria freshly isolated in clinical studies to determine their properties in different ecological situations. For example, we have recently demonstrated that strains of *Streptococcus mitior* could readily be isolated from approximal plaque from school children in a fluoridated area and shown to be inherently resistant to fluoride even at low pH (3). Organisms of this group were, in fact, isolated at lower pH in the presence of fluoride than were strains of *S. mutans*, an organism known to persist in certain naturally acidic plaque environments (13–15). In fact, the data suggested that *S. mitior*

would have a predominant niche in plaque communities continually exposed to fluoride in the water supply, particularly where the carbon supply was sufficient to reduce the environmental pH through the formation of metabolic acid. This suggests that *S. mitior* might, in fact, compete with *S. mutans* in the plaque ecosystem.

With this possible competition in mind, and recognizing that both pH and fluoride are important environmental parameters influencing the plaque in fluoridated areas, we undertook to compare the response of representative strains of *S. mitior* and *S. mutans* under conditions of pH and fluoride stress in continuous culture. Each organism was grown in complex medium with a glucose limitation and subjected to two periods of growth without pH control, one with the medium containing 50 μg of fluoride per ml. The results demonstrate that whereas *S. mitior* was more active glycolytically than *S. mutans*, the former organism could not survive at pH 5.1, and *S. mutans* could readily adapt to growth as low as pH 4.8. The addition of fluoride to the medium, although reducing the growth and metabolism of both organisms, nevertheless permitted the growth of *S. mitior* at a level that ensured its survival. Both cultures developed phenotypic resistance to high levels of fluoride (16 to 20 mM) during continuous growth in the fluoride-containing medium.

MATERIALS AND METHODS

Bacteriology. *S. mitior* 572 and *S. mutans* 2452 were isolated as previously described (3). Before the continuous culture experiments, the *S. mitior* isolate had received nine in vitro transfers, whereas the *S. mutans* strain had been transferred seven times since isolation from human dental plaque.

Growth conditions. Continuous growth was achieved in a New Brunswick chemostat (model C30) with a working volume of 375 ml. The chemostat was modified to include media flow through a variable-speed peristaltic pump (Watson-Marlowe, Falmouth, England). The medium inlet into the growth chamber passed through a heated tube held a 45°C with 3 × 50-Ω resistors coupled to a 25-W transformer and rheostat to prevent growth in the media inlet tube. The media contained the following (per liter): tryptone, 10 g; yeast extract, 5 g; KH_2PO_4, 1.33 g; K_2HPO_4, 2.66; $MgSO_4 \cdot 7H_2O$, 10 mg; $FeCl_2$, 10 mg; $MnSO_4 \cdot 4H_2O$, 10 mg; and NaCl, 10 mg. Glucose was at 2 mg/ml, and with this amount of glucose the growth of both organisms in the chemostat was limited by the carbon source.

General experimental design. The basic design of the continuous-culture experiments with both the *S. mitior* and *S. mutans* strains was as follows: (i) stage 1 was an initial phase of steady-state growth at pH 7.0; (ii) stage 2 was continuous growth without pH control; (iii) stage 3 was reestablishment of growth at pH 7.0; and, finally, (iv) stage 4 was a second period of continuous growth without pH control in media containing 50 μg of fluoride per ml. The dilution rate (D) with both organisms was 0.13 h^{-1} (mean generation time = 5.4 h) except that for *S. mitior* an additional run was carried out at D = 0.034 h^{-1} (mean generation time = 20 h) for comparison.

Each experiment was designed to monitor the following: (i) total viable cell counts and cell counts on fluoride-containing plates; (ii) routine maintenance of the chemostat including optical density, dry weight, and pH readings of the culture; and (iii) the glycolytic activity of washed cells obtained at each stage measured after incubation with glucose in a pH stat in the presence and absence of NaF.

Viable cell counts. Counts of the total numbers of viable cells in the medium from the chemostat chamber were made throughout the experiments. Dilutions of the medium up to 10^{-7} were made in reduced transport fluid (13). Portions, 20-μl, of these dilutions were spread onto blood agar plates (blood agar base no. 2, CM 271; Oxoid Canada, Toronto, Ontario) with 5% (vol/vol) sheep blood (Atlas Laboratories, Winnipeg, Manitoba) and onto a basal medium (3) at pH 6.5 and 6.0 with fluoride at 0, 10, 20, and 50 μg/ml. Duplicate plates were incubated at 37°C in 15% H_2–75% N_2–10% CO_2 for 16 h. Counts were made on plates viewed under a stereomicroscope on samples before and after sonication (3): the counts after sonication exceeded the counts on nonsonicated samples by a factor of 1.3 to 1.46, and the sonicated counts were recorded.

Routine maintenance of the chemostat. Daily routine maintenance included optical density readings in a Klett-Summerson spectrometer with a red filter (640 to 700 nm) and dry-weight measurements. The pH of the culture was also measured at least once a day, and the dilution rate was determined. Cell dry-weight determinations were carried out by filtering three 2- to 3-ml culture samples through preweighed 0.45-μm filters (Millipore Corp., Mississauga, Ontario). The glucose concentration in each batch of media and in the culture filtrate was determined by the glucose oxidase method of Kingsley and Getchell (12).

Assay for glycolytic activity. Cells for measurement of glycolytic activity were collected at 0°C from the overflow at various dilution rates for short time periods (1 to 5 h) and, on some occasions, in small volumes directly from the chamber. In the latter case, the amount collected never exceeded 10% of the chamber volume to minimize the perturbation of the culture. The cells were collected by centrifugation (10,000 × g for 10 min), washed in saline (0.85%), and resuspended at a concentration of 25 mg/ml; optical density versus dry-weight standard curves had been constructed for each strain (6). The glycolytic rate of this cell suspension was determined in a pH stat at constant pH (7.0) as previously described (10) except that the reaction mixture contained tryptone (10 g/liter) and yeast extract (5 g/liter) in 10 mM Na-K phosphate buffer (pH 7.0) to which was added 5 mg (dry weight) of cells in a total volume of 4.9 ml. The cell suspension was constantly mixed by a magnetic stirring bar in a stream of nitrogen gas, and the pH was kept constant with standardized 0.1 M NaOH added by means of a Radiometer Autoburette (model ABU 1a). The cell mixture was preincubated for sufficient time (usually 10 min) to run down the small endogenous reserves and traces of carbohydrate present in

the tryptone-yeast extract-buffer mixture. The glycolytic rate was then determined after the addition of 100 μl of 0.5 M glucose (final concentration, 12 mM).

This system was also used to test for the effect of NaF on the glycolytic rate at pH 7.0. Once the rate was established in the absence of NaF, 20- to 50-μl samples of NaF (100 mM) were added to the same reaction mixture in consecutive additions at suitable time intervals (usually 3 to 5 min), and the amount of NaOH required to maintain the pH at 7.0 was recorded for a specified time at each fluoride concentration. The fluoride concentration was calculated after considering the change in volume of the reaction mixture.

Throughout this work, 1 U of glycolytic activity is defined as 1 μmol of metabolic acid neutralized per mg (dry weight) of cells per min.

RESULTS

Growth and metabolic response of *S. mitior*. The growth of *S. mitior* 572 in continuous culture at $D = 0.13\ h^{-1}$ in complex medium with a glucose limitation at pH 7.0 gave cells numbers of $2.6 \pm 0.3 \times 10^9$/ml (Fig. 1A). On day 3, the pH control on the chemostat was turned off as stage 2 was initiated, and within 24 h the pH had dropped to 5.1 (Fig. 1B) and the total cell count had fallen to 9.6×10^6/ml. Since it was apparent on day 4 that the culture was "washing out" of the chemostat, pH control at 7.0 was resumed. After reestablishment of the culture at pH 7.0, cell numbers returned to near the original level at $2.4 \pm 0.6 \times 10^9$/ml within a 24-h period. For the final phase, stage 4, beginning at day 8, fluoride (50 μg/ml) was added to the medium and the pH control was again turned off. In contrast to stage 2, this resulted in a fall in cell numbers to only 9×10^7/ml within 24 h, and this held in a relatively constant fashion until the end of the experiment on day 21. This value was 10-fold higher than growth in the absence of fluoride during stage 2 as the culture was proceeding to wash out. The pH fell to 5.7 within 2 days and remained at that level for 4 days before falling to pH 5.4.

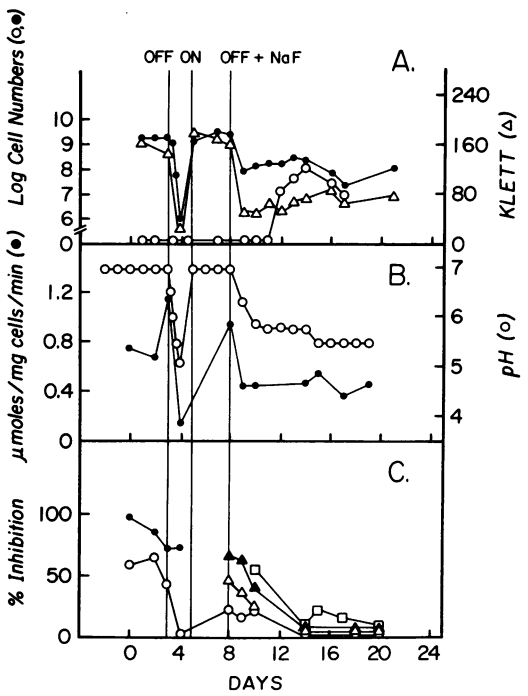

FIG. 1. Response of *S. mitior* 572 to changes in pH during growth in a chemostat in the presence and absence of NaF at $D = 0.13\ h^{-1}$. pH control was turned off on day 3, on again on day 4, and off on day 8, when 50 μg of fluoride per ml was added to the medium. (A) Total cell numbers on blood agar (●) and on agar plates at pH 6.5 containing 50 μg of NaF per ml (○). Klett optical density readings (△). (B) pH (○) and glycolytic rate (●) of resting cells collected from the chamber. (C) Inhibition (percent) of glycolysis by NaF: 2.2, ●; 3.9, ○; 4.8, △; 9, ▲; and 16 mM, □.

Glycolytic activity. Glycolytic activity was measured throughout the four stages of the experiment to test for the effect of pH and pH plus fluoride. In stage 1, high glycolytic activity was recorded, varying from 0.68 to 1.16 U (Fig. 1B). In the absence of pH control at stage 2, the glycolytic rate fell to 0.13 U as the pH fell to 5.1. On returning to pH 7.0 the glycolytic rate returned to 0.94 U, similar to that in the cells at stage 1. When pH control was again shut off, but with 50 μg of fluoride per ml in the medium, the rate fell only to 0.44 U and was maintained more or less at that level until the end of the experiment. Clearly, the presence of fluoride in stage 4 had maintained a more active culture in the absence of pH control when compared with stage 2.

The effect of fluoride was tested in two additional assays. Throughout the experiment cells were plated on agar at pH 6.5 and 6.0 in medium containing 0, 10, 20, and 50 μg of fluoride per ml. Although cells were shown to grow at pH 6.0 in the absence of fluoride, consistent growth at this pH in the presence of the inhibitor was not observed. However, whereas growth at pH 6.5 in the presence of 50 μg/ml was not observed during stages 1 to 3, cells capable of growing in the presence of fluoride at this concentration emerged during growth in stage 4 (Fig. 1A). This indicates that growth in the presence of fluoride generated resistant cells in significant numbers within 24 h of the addition of fluoride to the medium. Cells at this stage were phenotypically resistant since cells removed from the chemostat and grown in the absence of fluoride for several weeks were subsequently unable to grow at pH 6.5 and 50 μg of fluoride per ml.

The second assay involved testing the effect of various concentrations of fluoride on glycolytic activity by washed cells at each stage of the

experiment. Cells at stage 1 were severely inhibited by 3.9 mM NaF (Fig. 1C). Passage of cells through the low-pH period in stage 2 resulted in cells more resistant to NaF. For example, inhibition by 2.2 mM NaF fell from 40% on day 3 to 0% on day 4, and even during the next stage at pH 7.0 (stage 3) the cells retained a significant measure of their resistance. Entry into stage 4 (i.e., growth in the presence of 50 μg of fluoride per ml) resulted in the appearance of cells progressively more immune to the inhibitor.

A more complete comparison of the influence of various concentrations of fluoride on carbohydrate metabolism by *S. mitior* at various stages during the experiment can be seen in Fig 2A. Initially, glycolysis was severely inhibited by NaF at concentrations of <4 mM. However, as growth proceeded in the presence of NaF, metabolism became more insensitive to the inhibitor such that on day 19 only 2% inhibition was observed at a concentration of 16 mM NaF.

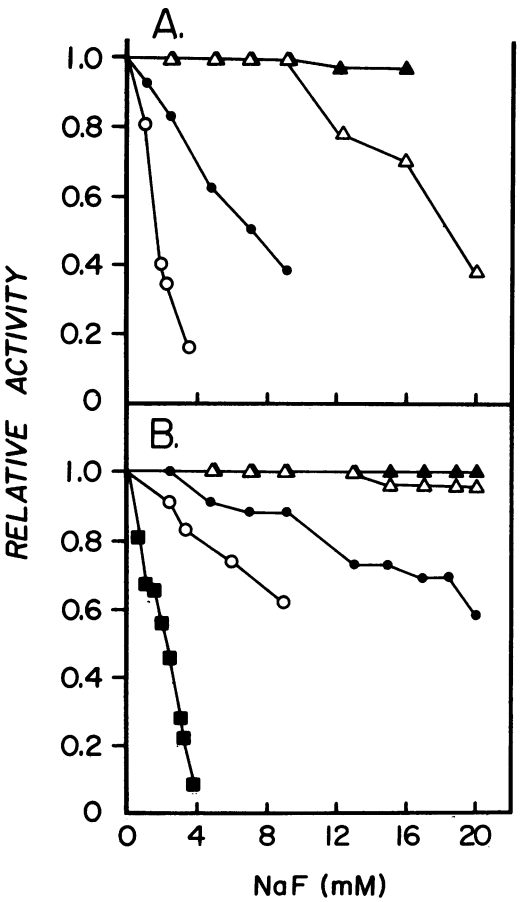

FIG. 2. Effect of NaF on the glycolytic rate by washed cells grown at $D = 0.13\ h^{-1}$ with a glucose limitation. (A) *S. mitior* 572: day 1 (no NaF), ○; day 9 (16 h with F in medium), ●; day 15, △; day 19, ▲. (B) *S. mutans* 2452: day 1 (no NaF), ○; day 21 (3 days with F in medium), ●; day 22, △; day 24, ▲. *S. mutans* DR0001 grown at $D = 0.1\ h^{-1}$ under identical conditions (■).

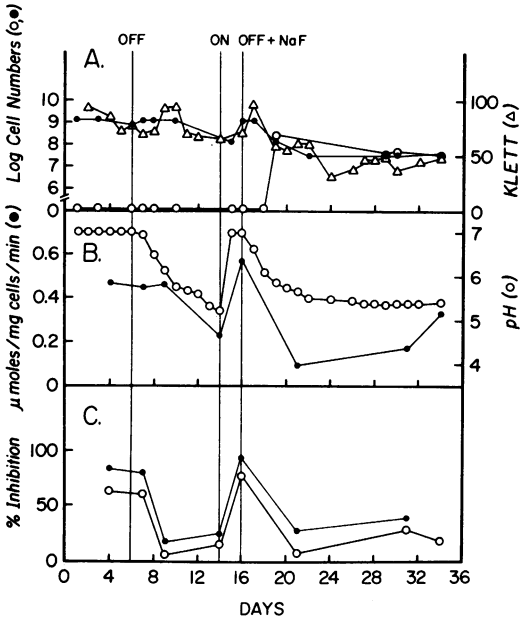

FIG. 3. Response of *S. mitior* 572 to changes in pH during growth in a chemostat in the presence and absence of NaF at $D = 0.034\ h^{-1}$; pH control was turned off on day 6, on again on day 14, and off on day 16, when 50 μg of fluoride per ml was added to the medium. See legend to Fig. 1 for symbols in (A), (B), and (C).

Effect of slow growth of *S. mitior*. To test for the effect of growth rate, *S. mitior* 572 was grown under conditions identical to those described in Fig. 1 but at $D = 0.034\ h^{-1}$ (mean generation time = 20 h). Under these conditions, rapid changes in pH and cell numbers at stages 2 and 4 were reduced by the very slow supply of medium (Fig. 3). At stage 1 during growth at pH 7.0, the total cell numbers were 1.8×10^9/ml, or some 30% lower than with the same culture growing at $D = 0.13\ h^{-1}$ (Fig. 1A). The differences between the yield of cells and the $Y_{glucose}$ for *S. mitior* at $D = 0.034$ and $0.13\ h^{-1}$ can be seen in Table 1. The faster growing cells gave values more than twice those for the same cells growing under the same conditions but at $D = 0.034\ h^{-1}$. Once the pH was shut off at $D = 0.034\ h^{-1}$, the cell numbers gradually fell to 1.5×10^8 at day 15 as the pH decreased to 5.2. Clearly, the loss of pH control at this growth rate was not as devastating as that observed at $D = 0.13\ h^{-1}$, although the pH minimum was nearly the same in both cases (i.e., 5.2 versus 5.1).

TABLE 1. Cell yields during growth of *S. mitior* 572 and *S. mutans* 2452 in a chemostat on complex medium with a carbon limitation[a]

Culture	D (h^{-1})	Yield of cells (mg/ml)	$Y_{glucose}$ (g of cells/mol of glucose)
S. mitior 572	0.034	0.30	27
	0.13	0.63	57
S. mutans 2452	0.13	0.82	74

[a] Residual glucose = 0.

Growth at stage 4 in the presence of NaF resulted in a reduction in cell numbers from 1.6 $\times 10^9$ to 3.5 $\times 10^7$/ml; however, cells resistant to 50 μg of fluoride per ml rapidly appeared in a fashion similar to that seen in Fig. 1C for cells growing at $D = 0.13\ h^{-1}$.

Glycolytic activity. Glycolytic activity by the culture mirrored the changes in the pH of the culture (Fig. 3B). Initially (stage 1) the glycolytic rate was 0.46 U, or about half the rate of the cells growing at $D = 0.13\ h^{-1}$. However, the rate fell to only 0.23 U during the period without pH control (stage 2), indicating that the cells were more active at pH 5.2 than those growing at pH 5.1 at the faster rate (i.e., 0.13 U). Conversely, once the pH had been reestablished at pH 7.0 and the pH control was again shut off with fluoride in the medium (stage 4), the glycolytic rate fell from 0.54 to 0.09 U within 5 days, although the pH did not fall below 5.4. With time, however, the cells adapted to fluoride and the glycolytic rate increased to 0.38 U at day 34.

The effect of NaF on glycolytic activity also reflected the changes in the culture pH (Fig. 3C). Cells growing in stages 2 and 4 at pH values below 6.0 showed increased resistance to fluoride, as seen with the cells growing at the higher dilution rate (Fig. 1C).

Growth and metabolic response of *S. mutans*. The growth of *S. mutans* 2542 in continuous culture at $D = 0.13\ h^{-1}$ under conditions identical to those for *S. mitior* (i.e., Fig. 1) showed some interesting differences in response to environmental change. With *S. mutans* (Fig. 4) the cell numbers were 20-fold higher during stage 1 (Fig. 4A) than those observed with *S. mitior* (i.e., 52.1 $\times 10^9$ versus 2.6 $\times 10^9$/ml), and the yield and $Y_{glucose}$ were 30% higher than those observed with *S. mitior* at the same dilution rate (Table 1).

The most interesting feature of this experiment was the response of *S. mutans* to an acid environment. When the pH control on the chemostat was shut off on day 6 (stage 2) the pH, like that for *S. mitior*, fell to 5.1 within 24 h (Fig. 4B). However, unlike this latter organism, *S. mutans* did not wash out of the chemostat, but the cell numbers fell from 41.0 to 3.8 $\times 10^9$/ml and the culture maintained itself at the latter value for 5 days, although the pH fell slowly to 4.8. Doubling the glucose concentration of the medium on day 12 resulted in a further drop in pH to 4.5 and a progressive fall in cell numbers to 2.1 $\times 10^8$/ml for the 3-day period, indicating that the culture was being stressed by the generation of additional metabolic acid.

The return of the culture to pH 7.0 (stage 3) followed by the final stage without pH control plus fluoride (stage 4) gave results similar to that seen with *S. mitior* in Fig. 1. Cell numbers fell to values of 3 $\times 10^8$ to 6 $\times 10^8$/ml in stage 4, and these numbers were maintained until the end of the experiment on day 28. As in the experiments with *S. mitior*, cells resistant to growth at pH 6.5 with 50 μg of fluoride per ml appeared within 24 h of the onset of stage 4.

Glycolytic activity. Initially (stage 1), cells of *S. mutans* 2452 possessed 40% less glycolytic activity (0.61 versus 0.86 U) than cells of *S. mitior* (Fig. 4B). However, the onset of stage 2 (no pH control) illustrated a fundamental difference between the two strains. The fall in pH from day 6 to 7 resulted in a 45% reduction in glycolytic activity, but as the culture maintained

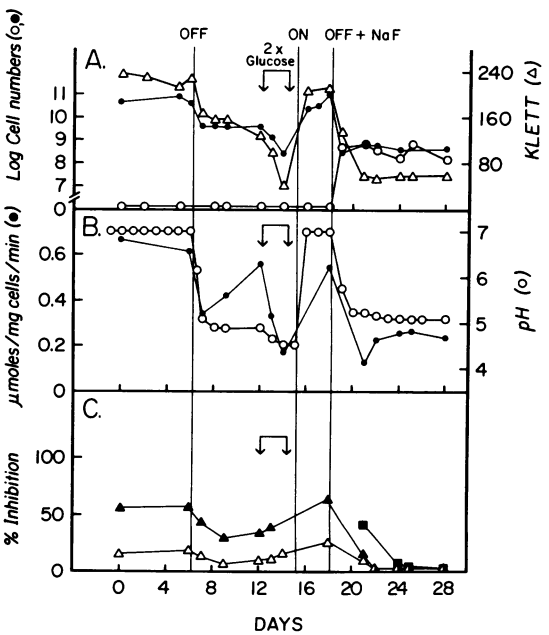

FIG. 4. Response of *S. mutans* 2452 to changes in pH during growth in a chemostat in the presence and absence of NaF at $D = 0.13\ h^{-1}$. pH control was turned off on day 6, on again on day 15, and off on day 18, when 50 μg of fluoride per ml was added to the medium. See legend to Fig. 1 for symbols for (A), (B), and (C). ■, 20 mM NaF.

itself at pH 4.8 the cells adapted to this environment such that the glycolytic rate increased from 0.34 to 0.55 U, indicating the adaptive and aciduric nature of the organism. The imposition of a twofold increase in glucose concentration in the media at day 12 resulted in cells increasingly deficient in glycolytic activity such that in 2 days the specific activity had decreased to 0.16 U as the cells were stressed by the low pH.

On reestablishing the culture at pH 7.0, the glycolytic rate returned to the normal range (i.e., 0.54 U). During stage 4 (no pH control plus fluoride in the medium), the rate fell to 0.13 U and increased slightly with time to 0.27 U at day 24.

By comparing the effect of NaF on glycolytic activity by cells of *S. mitior* 572 (Fig. 1C) and *S. mutans* 2452 (Fig. 4C), one can see that the latter cells were inherently less fluoride sensitive since 9.1 mM NaF was required to inhibit glycolysis with *S. mutans* at stage 1 whereas only 2.4 mM NaF was required for the same degree of inhibition with *S. mitior*. As in the other experiments, the cells growing at low pH in stages 2 and 4 had increased fluoride resistance. For example, in stage 4, as growth continued in the presence of NaF from day 18 the degree of fluoride resistance increased such that by day 25 cells were able to metabolize glucose without inhibition at concentrations of NaF to 20 mM.

A comparison of the effect of various concentrations of NaF on the glycolytic activity of *S. mutans* throughout the experiment can be seen in Fig. 2B. During steady-state growth at pH 7.0 in stage 1, cells were inhibited 38% by 9 mM NaF and showed more resistance to NaF than did *S. mitior* at the same stage (Fig. 2A). However, as the period of growth in the presence of fluoride (50 μg/ml) increased in stage 4, resistance increased such that at day 24 the cells were completely immune to 20 mM NaF. Furthermore, *S. mutans* 2542, freshly isolated with only seven transfers in vitro, was shown to be inherently more fluoride resistant than *S. mutans* strain DR0001 transferred in vitro for several years and grown in the chemostat at $D = 0.1\ h^{-1}$ under conditions identical to those for *S. mutans* 2452.

DISCUSSION

Clearly, the chemostat cannot simulate the oral cavity, and the intent of the experiments reported here was not to extrapolate our findings in that fashion. However, growth of an organism in a chemostat can be used to predict the response of the organism to an environmental change that might occur in nature. By establishing the response of various predominant oral bacteria under a series of controlled and reproducible experiments, one should establish a clearer picture of the nature of important oral ecosystems.

The two organisms used in these experiments exhibited some fundamental differences which might assist us in predicting their relative niche in the plaque ecosystem. For example, although *S. mitior* is present in plaque in relatively high numbers (3) and is glycolytically very active (Fig. 1), it is, nevertheless, highly sensitive to an acid environment. In other words, although it is highly acidogenic, it is not aciduric and would be reduced or even eliminated in a habitat receiving a relatively steady supply of fermentable carbohydrates or of a structure such as to prevent ready neutralization of acid by the buffers in saliva. *S. mutans* being aciduric, on the other hand, would resist elimination in this environment provided that the amount of carbohydrate present in the environment was not sufficiently high to reduce the pH below 4.8. Certainly, the test strain of *S. mutans* was not greatly affected by an environment from pH 7.0 to 4.8 and would adapt its metabolism to the acid environment. Thus, like all oral ecosystems, there is a balance between substrate concentration and the pH generated by metabolism, with *S. mitior* operating in a higher pH range than *S. mutans* (Fig. 5). It is perhaps no coincidence that the operating pH range (pH 7.0 to 4.8) for the latter organism is more coincident with that pH resulting in enamel demineralization leading to dental caries (5).

The application of fluoride to the system resulted in two often forgotten effects. Whereas it

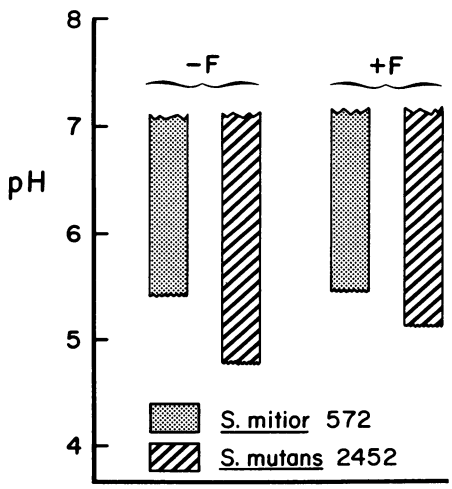

FIG. 5. Approximate viability zone in the acid region for *S. mitior* 572 and *S. mutans* 2452 during growth in a chemostat at $D = 0.13\ h^{-1}$ with a glucose limitation in the presence and absence of 50 μg of fluoride (F) per ml.

is generally recognized that fluoride will inhibit glycolysis, it is not often remembered that organisms, particularly oral streptococci, acquire resistance to high concentrations of fluoride (8). The exact nature of this fluoride resistance is unknown, and both phenotype (17) and genotype (7) resistance to fluoride has been observed. The resistance observed in the experiments reported here was generated in response to two types of stimuli: (i) growth at low pH (stage 2) and (ii) a more pronounced form of resistance in response to the presence of NaF in the growth medium (stage 4).

Stage 2 resistance developed through the need of each culture to grow at pH values approaching 5.0, and this form of resistance has been observed during continuous-culture experiments with *S. mutans* Ingbritt (9). Growth of the latter organism at pH 5.5 resulted in the repression of the phosphoenolpyruvate phosphotransferase system for the transport of glucose, although glycolysis was stimulated threefold compared with its value with growth at pH 6.5. Associated with this increased activity was a concomitant increase in fluoride resistance. The glucose-phosphotransferase system is sensitive to fluoride through the inhibition of the phosphoenolpyruvate-generating enzyme enolase (8). This earlier result, therefore, demonstrated the emergence of a non-phospotransferase transport system relatively immune to fluoride. Recent results with a mutant of *S. mutans* 6715-10 (11), now designated DR0001, has indicated that the alternate system is driven by proton motive force. Thus, with *S. mitior* 572 and *S. mutans* 2452, it is likely that the stage 2 fluoride resistance was a reflection of a shift from a more fluoride-sensitive to a less fluoride-sensitive transport system as the culture shifted from growth at neutrality to growth in an acid environment.

Stage 4 resistance was a much more pronounced change since both organisms adapted to the point where glycolysis was inhibited only slightly, or not at all, by 20 mM NaF. The exact nature of this resistance is unknown; however, it appears to be transitory (phenotypic) since the resistance is lost during growth of the organisms in fluoride-free medium. It is interesting to note that the freshly isolated test strains were more inherently fluoride resistant than an *S. mutans* strain, DR0001 (Fig. 2B), transferred in the laboratory for many months.

An important ecological feature is also demonstrated with fluoride present in the culture environment. Whereas the growth and metabolism of *S. mutans* is reduced by fluoride, the fluoride ion is also responsible for the survival of *S. mitior* in a carbohydrate-rich environment at low pH. The inhibition of glycolysis in the latter organism by fluoride resulted in less acid being formed (i.e., pH 5.4 versus 5.1), and in vivo this could aid in stabilizing the organism in the community. One could readily envisage such phenomena occurring on the tooth surface where various populations of acidogenic, nonaciduric bacteria exist. Fluoride could act to stabilize the community and in doing so remove any ecological advantage from the aciduric populations present in the habitat. Such a stabilizing effect might allow levels of competition and growth resembling more closely those existing in ecosystems with little or no carbohydrate. Adaptation of aciduric strains to produce more acid in the presence of fluoride may negate this effect if carbohydrate intake is excessive. Such a competitive effect would depend upon the extent of the diffusion of acid within the ecosystem.

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