The Effect of Lowering the pH on the Composition and Metabolism of a Community of Nine Oral Bacteria Grown in a Chemostat

By ANN S. McDERMID,† AILSA S. McKEE,† D. C. ELLWOOD AND
P. D. MARSH*†

*Pathogenic Microbes Research Laboratory, PHLS Centre for Applied Microbiology and
Research, Porton Down, Salisbury, SP4 0JG, UK*

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Nine oral bacteria, associated with both healthy and diseased sites in the mouth, were grown at $D = 0.05 \text{ h}^{-1}$ (mean generation time 13.9 h) in a glucose-limited chemostat. After an initial period of steady-state growth at pH 7.0, pH control was discontinued. The pH then decreased until it stabilized at pH 4.1 after 9 d (16 generations), while the E_h rose from -165 mV to $+160 \text{ mV}$. The lowering in pH resulted in the composition and metabolism of the flora being altered and in increased bacterial aggregation. At pH 7.0, '*Streptococcus mitior*', *Veillonella alcalescens* and *S. sanguis* were most numerous while at pH 4.1 the counts of all bacteria fell except for *Lactobacillus casei*, which became predominant. The proportions of *S. mutans* within the community also increased while *S. sanguis* was recovered only occasionally and *Bacteroides intermedius* was not detected below pH 4.6. The survival at pH 4.1 of several other species would not have been predicted from earlier pure culture studies. Relative to pH 7.0, the community growing at pH 4.1 produced more lactic acid, washed cells had a greater glycolytic activity over a wider pH range but amino acid metabolism decreased. In general, when pH control was restored, so were the original patterns of metabolism and bacterial counts, except for *B. intermedius*, which was still not detected. The inverse relationship between *S. sanguis* and *S. mutans*, and the increase in proportions of *L. casei* and *S. mutans* during growth in a low pH environment parallel observations made *in vivo* and suggest that the chemostat can be used as a model for microbial behaviour in dental plaque.

INTRODUCTION

Dental plaque is the complex microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. It can be found on healthy enamel surfaces but is also implicated in the aetiology of two of the most prevalent diseases affecting industrialized societies: caries and periodontal disease. The transition of the oral flora from a commensal to a pathogenic relationship with the host is associated with a change in the metabolism and composition of the flora at specific sites (for a review, see Marsh & Martin, 1984). Owing to the complexity of the oral ecosystem, it has proved difficult to identify the ecological factors responsible for such changes. Consequently, a number of *in vitro* models have been developed of which the chemostat is of particular value (van der Hoeven & de Jong, 1984). This is because bacteria grow in plaque at sub-maximal rates (Beckers & van der Hoeven, 1982) under a range of possible carbohydrate concentrations, nutrient limitations and pH values, all of which can be readily studied in isolation using conventional continuous culture techniques. Although the chemostat provides a homogeneous environment, complex communities of oral bacteria have been maintained for long periods under different environmental conditions when

† Present address: Bacterial Metabolism Research Laboratory, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 0JG, UK.

plaque was used as the inoculum (Brown *et al.*, 1978; Marsh *et al.*, 1983). However, in order to compensate for the known variability in the composition of plaque taken from different sites or even from the same site at different times, recent chemostat studies have been standardized by the use of a defined inoculum of nine representative oral bacteria (McKee *et al.*, 1985). In this latter study, the bacterial composition and metabolic activity of the communities were similar when grown under identical conditions in replicate experiments. Using the chemostat in this way it should be possible to observe simple cause-and-effect relationships after the alteration of a single variable. In the aetiology of dental caries, the growth and metabolism of bacteria at low external pH values are of prime importance. These low pH values occur regularly in plaque after the ingestion and metabolism of dietary sugars; for an example, see Schachtele & Jensen (1982). Consequently, we have studied the effect of lowering the pH on the behaviour of a community of oral bacteria growing initially at pH 7.0.

METHODS

Bacterial strains. Nine organisms were chosen for their known importance and prevalence in dental plaque in both health and disease, and also for their relative ease of isolation and identification (McKee *et al.*, 1985). The bacteria were *Veillonella alcalescens* ATCC 17745; *Streptococcus mutans* ATCC 27351 (a streptomycin-resistant strain); *Streptococcus sanguis* NCTC 7865; '*Streptococcus mitior*' EF 186 (this strain does not produce extracellular polysaccharides from sucrose); *Actinomyces viscosus* WVU 627; *Bacteroides intermedius* (previously *Bacteroides melanogenicus* subsp. *intermedius*) T588; *Fusobacterium nucleatum* ATCC 10953; *Neisseria* sp. A1076 and *Lactobacillus casei* AC 413. The non-culture-collection strains were human dental plaque isolates obtained from J. M. Hardie and H. N. Shah, Department of Oral Microbiology, The London Hospital Medical College, London E1 2AD.

Growth conditions. The chemostat was operated at a dilution rate (D) of 0.05 h^{-1} (mean community generation time 13.9 h), a temperature of $37\text{ }^{\circ}\text{C}$, a working volume of 600 ml, and under a gas phase of 5% CO_2 in N_2 . The pH was maintained initially at 7.0 ± 0.1 by the automatic addition of 2 M-NaOH. The E_h was measured *in situ* with an autoclavable electrode; the standard used to calibrate the electrode was saturated quinhydrone (BDH) in 0.1 M-HCl (Russell & Coulter, 1975). The chemostat growth medium was a modification of BM medium (Shah *et al.*, 1976), composition per litre distilled water: proteose peptone (Oxoid), 10 g; trypticase peptone (BBL, Becton Dickinson), 5 g; yeast extract (Difco), 5 g; potassium chloride, 2.5 g; cysteine hydrochloride (BDH), 0.5 g; haemin (Sigma), 5 mg; vitamin K_1 (Sigma), 1 mg. The medium was brought to the boil and sterilized by autoclaving at $121\text{ }^{\circ}\text{C}$ for 30 min.

To achieve glucose limitation, this modified BM medium was supplemented with 28 mM-sterile glucose (McKee *et al.*, 1985). After a period of steady-state growth at pH 7.0, the pH controller was switched off and the pH was allowed to stabilize. Once stabilized, and after a period of steady-state growth, pH control was restored and the pH was returned to and maintained at 7.0 ± 0.1 . Ten pot volume changes of media (equivalent to 14 community generations) were allowed to elapse before a culture was deemed to have reached a steady state.

Chemostat inoculation. Each strain was grown anaerobically (except the *Neisseria* sp, which was grown aerobically) in batch culture to late exponential phase in BM medium containing 55.6 mM-glucose (*V. alcalescens* was grown in BM medium containing 122.7 mM-sodium lactate). From each culture 20 ml was mixed and 90 ml of this mixture inoculated into a chemostat. The purity of the inoculum was confirmed and viable counts made using a range of selective and non-selective media (McKee *et al.*, 1985). Then 200 ml culture medium was pumped into each chemostat to enable pH and temperature to be controlled and D was adjusted to 0.05 h^{-1} . A previous study had shown that not all of the nine bacteria are able to colonize after the first inoculation (McKee *et al.*, 1985). Consequently, the chemostat was re-inoculated with all nine bacteria on two further occasions, 3 and 5 d after the first inoculation. Before each re-inoculation, a 100 ml volume of culture was removed from each chemostat to accommodate the fresh 90 ml inoculum and to ensure that the flow of medium was uninterrupted.

Viable counts of chemostat cultures. Viable counts of the microbial communities of each chemostat were made daily for at least the first 14 days, then thrice weekly until termination of the experiments. Samples were removed from the chemostats, dispersed, serially diluted in freshly-reduced BM medium and plated on a range of selective and non-selective media (McKee *et al.*, 1985). All plates were incubated anaerobically for 6 d in an atmosphere of 10% (v/v) CO_2 , 20% (v/v) H_2 in N_2 , except for one set of vancomycin blood agar plates which were incubated aerobically for 3 d for the enumeration of *Neisseria* sp. Identification was assessed by colonial morphology, growth on selective media, Gram-staining reaction and on catalase production (McKee *et al.*, 1985).

Washed cell experiments. Cells were collected during steady-state conditions via the chemostat overflow into a container cooled in ice, and were harvested by centrifugation (8000 g for 10 min). Cells were washed twice by centrifugation (8000 g for 10 min), resuspended in 200 mM-KCl to give approximately 30 mg (dry weight) cells ml^{-1} and stored in ice until used. The washed cells were then diluted in 200 mM-KCl to give a final concentration of

5 mg (dry weight) ml⁻¹ in 5 ml. Acid production from storage material (endogenous metabolism) or after a pulse of excess (3 mM) glucose, sucrose, fructose or lactose was measured at 37 °C under a constant flow of O₂-free N₂ in a pH-stat (Radiometer) either as a pH-fall from pH 7.0, in which the terminal pH was recorded after 15 min, or as a glycolytic rate at a constant pH by the automatic titration of neutralizing alkali (100 mM-KOH) (Marsh *et al.*, 1982). Units of glycolytic activity are expressed as nmol acid neutralized (mg dry weight cells)⁻¹ min⁻¹.

Analytical procedures. The E_h , turbidity, dry weight and microscopic appearance of the cultures were determined regularly. During steady-state conditions the residual glucose, acid end products of metabolism and amino acid composition of culture filtrates were determined as described previously (Marsh *et al.*, 1983).

RESULTS

Bacterial composition and metabolic properties of the microbial community growing initially at pH 7.0

After the chemostat had been inoculated on three separate occasions, all of the nine organisms present in the inoculum became established in the chemostat at pH 7.0 although the proportions of individual populations varied markedly. The predominant species were '*S. mitior*' and *V. alcalescens* while *Neisseria* sp. was present in only low numbers (Table 1). No residual glucose could be detected in culture filtrates (Table 2) and neither could arginine, cysteine, lysine and tryptophan (Table 3). In contrast, several other amino acids, and ornithine in particular, increased in concentration. Under steady-state conditions, the community of bacteria produced a mixed pattern of fermentation products with only low concentrations of lactic acid (Table 4), and the E_h of the culture remained relatively stable at approximately -165 mV (Fig. 1).

Bacterial composition and metabolic properties of the microbial community growing at pH 4.1

When pH control of the culture was stopped, the pH fell rapidly to 5.7 and within two community generations it had reached 4.8. It finally stabilized at pH 4.1 nine d (16 community generations) after the pH controller was switched off; during this period, the E_h of the culture rose to approximately +160 mV (Fig. 1). Eight of the nine organisms survived the fall in pH and remained at relatively stable levels during growth at the lower pH (Table 1), although their ranking order with respect to one another and their viable counts changed. At pH 4.1, the predominant organisms under steady-state conditions were *L. casei*, *V. alcalescens* and *S. mutans*. Except for *L. casei*, the counts of all species fell and *S. sanguis* was recovered only twice (and in low numbers) (Fig. 1, Table 1); *B. intermedius* was not detected below pH 4.6. During steady-state conditions, residual glucose could be detected in culture filtrates (Table 2) and there was a reduced pattern of amino acid metabolism (Table 3). The pattern of fermentation products also changed and lactic acid was present in the highest concentrations (Table 4). The yield (dry weight) and total viable count of the community growing at pH 4.1 were markedly lower than at pH 7.0 (Table 2); bacterial aggregates could also be seen more commonly during growth at pH 4.1.

Bacterial composition and metabolism of the bacterial communities when the pH was returned to pH 7.0

When pH control was restored, the E_h fell as the pH returned immediately to neutrality (Fig. 1). There was a rapid increase in the numbers of several bacterial populations and, when steady-state conditions were attained, the viable counts and ranking order for many species were similar to their original values (Table 1). However, *B. intermedius*, which was lost from the culture when the pH fell, did not re-establish and counts of *Neisseria* sp., after an initial rise (Fig. 1), remained low. In contrast, *S. sanguis*, which persisted in barely detectable numbers at pH 4.1, was present at levels even higher than those obtained originally at pH 7.0 (Table 1). This rise in *S. sanguis* was accompanied by a slight fall in the numbers of *S. mutans* compared to its original count at pH 7.0. The general patterns of fermentation products (Table 4), amino acid metabolism (Table 3) and glucose utilization (Table 2) together with the total viable count of the community were similar to those obtained originally at pH 7.0, although the yields were lower (Table 2).

Table 1. *Bacterial composition of a community of oral bacteria growing at different pH values*

The bacterial community was grown initially at pH 7.0. After a period of steady-state growth, pH control was stopped and the pH eventually stabilized at pH 4.1; the pH was then returned to neutrality. Viable counts (c.f.u. ml⁻¹) were determined on a range of selective and non-selective media as described in Methods. All counts were determined when cultures were in steady state. Numbers in parentheses are the sample size; ND, not detected.

Bacterial population	Viable count [mean log ₁₀ (c.f.u. ml ⁻¹) ± SD]		
	Initial pH 7.0	pH 4.1	Final pH 7.0
<i>S. mitior</i> *	8.6 ± 0.2 (5)	4.2 ± 0.7 (12)	8.6 ± 0.5 (4)
<i>S. mutans</i>	6.1 ± 0.7 (5)	4.8 ± 0.3 (14)	5.6 ± 0.2 (4)
<i>S. sanguis</i>	7.5 ± 0.3 (5)	+	8.4 ± 0.2 (4)
<i>A. viscosus</i>	6.4 ± 0.4 (5)	4.1 ± 1.1 (12)	6.0
<i>L. casei</i>	7.4 ± 0.2 (4)	7.5 ± 0.3 (16)	7.8 ± 0.4 (4)
<i>Neisseria</i> sp.	3.6 ± 0.5 (3)	1.3 ± 0.4 (14)	1.0 ± 0 (3)
<i>V. alcalescens</i>	8.2 ± 0.1 (6)	5.7 ± 0.3 (16)	8.2 ± 0.1 (4)
<i>B. intermedius</i>	7.4 ± 0.3 (5)	ND	ND
<i>F. nucleatum</i>	7.3 ± 0.3 (5)	1.4 ± 0.4 (9)	8.1 ± 0.5 (4)

* Present but detected only occasionally and in low numbers.

Table 2. *Yields of a microbial community of oral bacteria grown in a chemostat at different pH values*

H of culture	Dry wt (mg ml ⁻¹)*	Residual glucose (mg ml ⁻¹)*	Glucose consumed (mg ml ⁻¹)†	Y _{glucose} [mg dry wt cells (mg glucose consumed) ⁻¹]	Y _{molar glucose} [g dry wt cells (mol glucose consumed) ⁻¹]	Total viable count [mean log ₁₀ (c.f.u. ml ⁻¹)]*
Initial pH 7.0	1.87 ± 0.04 (2)	0 (2)	5.0	0.37	67.3	8.86 ± 0.43 (3)
pH 4.1	0.25 ± 0.09 (3)	3.3 ± 0.3 (3)	1.7	0.15	26.5	7.58 ± 0.10 (5)
Restored to pH 7.0 from 4.1	1.37 ± 0.31 (5)	0 (3)	5.0	0.27	49.3	8.98 ± 0.64 (6)

* Results are ±SD; numbers in parentheses are the sample size.

† The glucose concentration in the uninoculated medium was 5.0 mg ml⁻¹.

Table 3. *Metabolism of amino acids during steady-state conditions in a chemostat by a community of oral bacteria grown at different pH values*

The metabolism is expressed as a percentage increase or decrease in the concentration of the amino acid in culture supernatants compared with the uninoculated medium. The results are the mean of at least duplicate samples taken from the chemostat on different days.

Amino acid	Concn in uninoculated medium (mM)	Percentage increase (+) or decrease (−) in amino acid concn		
		Initial pH 7.0	pH 4.1	Final pH 7.0
Alanine	2.8	+200	0	+100
2-Aminobutyric acid	—	+	+	+
Arginine	1.7	−100	−17	−100
Aspartic acid	1.9	−80	0	−64
Citrulline	—	+	0	0
Cysteine	1.1	−100	−22	−100
Glutamic acid	2.7	−97	0	−96
Glycine	2.1	+200	+5	+116
Histidine	1.9	−88	−30	−93
Isoleucine	1.6	+71	+17	+100
Leucine	3.6	+25	0	+25
Lysine	2.2	−100	0	−100
Methionine	1.1	−90	0	−70
Ornithine	0.1	+1000	+567	+1000
Phenylalanine	1.7	+53	+14	+30
Proline	0.3	+500	0	+420
Serine	1.8	−91	0	−84
Threonine	1.3	−42	0	−50
Tryptophan	0.3	−100	0	−100
Valine	1.6	+128	0	+100

* Detected in culture filtrates but not present in the uninoculated medium.

Table 4. *Fermentation products produced during steady-state growth in a chemostat of a community of oral bacteria growing at different pH values*

Numbers in parentheses are the sample size.

Fermentation product	Concn (mM) $\pm$ SD		
	Initial pH 7.0	pH 4.1	Final pH 7.0
Ethanol	16.5 $\pm$ 3.2 (2)	17.3 $\pm$ 8.7 (5)	20.4 $\pm$ 4.3 (3)
Acetic acid	81.2 $\pm$ 1.8 (2)	7.3 $\pm$ 4.9 (6)	89.2
Propionic acid	17.3 $\pm$ 0.2 (2)	0.3 $\pm$ 0.2 (6)	22.9 $\pm$ 6.3 (3)
iso-Butyric acid	0 (2)	0.1 $\pm$ 0.1 (6)	0 (3)
n-Butyric acid	14.8 $\pm$ 0.2 (2)	0.1 $\pm$ 0.1 (6)	14.6 $\pm$ 3.7 (3)
iso-Valeric acid	0.3 $\pm$ 0.3 (2)	0.1 $\pm$ 0.1 (6)	0.2 $\pm$ 0.2 (3)
Formic acid	36.0 $\pm$ 7.0 (2)	0 (5)	36.1 $\pm$ 0.1 (2)
Lactic acid	0.4 $\pm$ 0.02 (2)	56.8 $\pm$ 10.7 (5)	0.6 $\pm$ 0.1 (2)

Acid production by washed cells harvested from bacterial communities growing at pH 7.0 and pH 4.1

The community growing at pH 4.1 had a higher level of endogenous metabolism of storage compounds and made more acid after a pulse of glucose, fructose or lactose than did the community growing at pH 7.0, either before or after the pH change (Table 5). The exception was with sucrose and this probably reflected the bacterial composition of the communities at the different pH values for the following reason. The strain of *L. casei* used in this study, and the predominant organism at pH 4.1 (Table 1), can not metabolize sucrose (Marsh *et al.*, 1984). Acid from glucose was also made at a faster rate and over a wider pH range by the community grown

Fig. 1. Effect of pH on the E_h and bacterial composition of a community of nine oral bacteria growing in a chemostat. Viable counts [$\log_{10}$ (c.f.u. ml^{-1})] of (a) '*S. mitior*' ($\circ$), *S. sanguis* ($\bullet$) and *S. mutans* ($\blacktriangle$); (b) *Neisseria* sp. ($\blacksquare$), *V. alcalescens* ($\square$), *B. intermedius* ($\blacklozenge$) and *F. nucleatum* ($\diamond$); (c) *L. casei* ($\blacktriangledown$) and *A. viscosus* ($\triangle$). After a period of steady-state growth at pH 7.0, pH control was discontinued (filled arrow) and the pH was regulated by the metabolism of the flora (d, solid line). When the pH had stabilized and steady-state conditions had been reached, pH control was re-introduced (open arrow) and returned immediately to pH 7.0. The E_h of the culture was monitored throughout this period (d, dashed line).

at pH 4.1 (Fig. 2). Its activity at pH 4.0 was greater than that obtained at any pH value by the communities growing at pH 7.0. The pH optimum for acid production also shifted to a lower value compared to the communities grown at neutral pH (Fig. 2). Acid production by the community growing when the pH was returned to 7.0 was similar to the values obtained before the pH controller was switched off (Table 5, Fig. 2).

DISCUSSION

Ecological studies of microbial films such as dental plaque have been hindered because the repeated sampling of specific sites in man or animals, over short intervals, leads inevitably to the physical disruption of the sample area, which might, in itself, lead to a change in the bacteriological or biochemical profile of the flora. Consequently, a chemostat model has been developed whereby a representative community of oral bacteria can be grown reproducibly for long periods from a defined inoculum (McKee *et al.*, 1985). The effect of varying single parameters on the behaviour of these communities can therefore be monitored regularly with ease.

Fig. 2. Glycolytic rates of washed cells of a community of oral bacteria grown in a chemostat at different pH values. Cells were harvested from the chemostat and washed in 200 mM-KCl, and glycolytic activity after a pulse of glucose was determined in a pH-stat at different pH values. Glycolytic activity was measured as amount (nmol) of alkali required to neutralize metabolic acid (mg dry weight cells)⁻¹. Cells were harvested under steady-state conditions when the pH of the chemostat was initially at pH 7.0 (○), at pH 4.1 (▲) and when it was returned to pH 7.0 (●). The results are the mean of triplicate values using cells collected on different days.

Table 5. *Acid production by harvested oral communities growing in a chemostat under steady-state conditions at different pH values*

Cells were harvested, washed and resuspended in 200 mM-KCl at pH 7.0. Acid production from endogenous metabolism of storage material or after a pulse of excess (3 mM) glucose, sucrose, fructose or lactose was measured as the terminal pH after 15 min. Numbers in parentheses are the number of estimations made on different days with freshly prepared cells.

Substrate	Terminal pH after 15 min ± SD		
	Initial pH 7.0	pH 4.1	Final pH 7.0
Storage material	6.75 ± 0.05 (2)	5.84 ± 0.40 (4)	6.80 ± 0.29 (2)
Glucose	5.24 ± 0.13 (2)	4.08 ± 0.18 (4)	5.26 ± 0.28 (5)
Sucrose	5.54 ± 0.06 (2)	5.83 ± 0.37 (4)	5.58 ± 0.54 (5)
Fructose	6.49 ± 0.04 (2)	5.08 ± 0.29 (3)	5.92 ± 0.45 (3)
Lactose	6.44 ± 0.02 (2)	6.07 ± 0.37 (2)	6.47 ± 0.62 (2)

All nine bacterial populations present in the inoculum grew at pH 7.0. This corresponds approximately to the resting pH of plaque on healthy enamel surfaces (Geddes & Jenkins, 1974). The composition and behaviour of this community was also consistent with what may be expected at healthy sites exhibiting, for example, high levels of amino acid metabolism (Table 3), a mixed pattern of fermentation products with lactate present as only a minor component (Table 4), and only small changes in pH resulting from the endogenous metabolism of storage material or after a sugar pulse (Table 5). When pH control of the chemostat was discontinued, the pH fell rapidly at first and then more slowly, and finally stabilized at pH 4.1. This was accompanied by a simultaneous rise in E_h ; a similar inverse relationship was reported previously in a study of films of oral micro-organisms grown in an artificial mouth (Russell & Coulter, 1975). Surprisingly, except for *B. intermedius*, which was not detected below pH 4.6, all of the bacteria persisted under steady-state conditions at pH 4.1. This would not have been predicted from recent pure culture studies in which a number of oral bacteria including *S. sanguis* and *A. viscosus* could not grow at pH 5.0 (Harper & Loesche, 1984). This again emphasizes the need for caution when extrapolating from pure culture studies in the laboratory to complex, mixed culture systems *in vivo*. Other studies, using an artificial mouth, have also shown that some bacteria, such as *A. viscosus*, are better able to survive in a complex mixed culture rather than simple mixtures of two organisms, even at low pH (Ahmed & Russell, 1978).

The apparent high acid tolerance of the bacteria reported here might be a consequence of the pH being lowered gradually by the metabolism of the bacteria themselves, thus giving an opportunity for the organisms to adapt, or it may suggest that communities of interacting bacteria can protect one another from the effects of low pH environments. Certainly, aggregation between cells was observed during growth at this low pH although the bacteriological composition of these associations was not studied. In an earlier study of oral bacteria grown in mixed continuous culture at pH 7.0, co-aggregation between different species in the culture vessel was common, and obligately anaerobic bacteria (fusobacteria) were seen and recovered viable from aerobically-grown colonies of *Neisseria* (McKee *et al.*, 1985). In the present study, the survival of obligately anaerobic populations such as *V. alcalescens* and *F. nucleatum* during growth at pH 4.1 implied that the loss of *B. intermedius* was due to pH and not to the concomitant rise in E_h , although it is possible that these strains might have a differential sensitivity to E_h .

Although the resting pH of plaque is around neutrality, the pH falls to below pH 5.0 within minutes of the ingestion of dietary carbohydrates (Schachtele & Jensen, 1982), while the pH at the base of a carious lesion is frequently around 4.0, and may be as low as 3.2 (Dirksen *et al.*, 1963). In our study, when the pH fell to pH 4.1, with the exception of *L. casei*, the viable count of each species fell, although the numbers of *S. mutans* were less affected than those of other populations, particularly *S. sanguis* and *F. nucleatum* (Table 1, Fig. 1). Indeed, the proportions of *L. casei* and *S. mutans* within the community increased during growth at pH 4.1. Similar effects have been observed *in vivo* in man during the progression of incipient carious lesions (for example, see Loesche & Straffon, 1979; Marsh *et al.*, 1980; Boyar & Bowden, 1985), and after the regular rinsing of a low pH buffer solution (pH 3.9) around the oral cavity (Svanberg, 1980). In the latter study, the proportion of *S. mutans* fell to near its original level when use of the mouthrinse was discontinued, just as we found when pH control was restored to the chemostat.

Two bacterial populations that did not thrive at the lower pH were *F. nucleatum* and *B. intermedius*. Elevated levels of these organisms have been associated with some periodontal diseases; the pH of a periodontal pocket varies with its depth (Kleinberg & Hall, 1969) but is invariably above pH 7.0 and is commonly as high as pH 8.5 (Bickel & Cimasoni, 1985; Kleinberg & Hall, 1969). Thus, pH may be an important factor controlling the numbers of these organisms in plaque. In contrast, *V. alcalescens* grew well and remained a predominant organism at pH 4.1. *Veillonella* cannot metabolize sugars but can form food-chains with lactate-producing bacteria (Mikx & van der Hoeven, 1975) and, after studies with gnotobiotic animals (Mikx *et al.*, 1972), it was proposed that these organisms might modify the carious process by converting lactic acid to weaker acids (acetic and propionic). However, the recent findings of increased levels of *Veillonella* at carious sites in man (Boyar & Bowden, 1985; Milnes & Bowden, 1985), coupled with the *in vitro* observations that members of this genus can stimulate the growth (McKee *et al.*, 1983) and glycolytic activity (Hamilton & Ng, 1983) of *S. mutans*, suggest that the role of these bacteria in human plaque may have to be re-assessed. Certainly, the results presented here support the proposal that high levels of *Veillonella* might reflect environments with a high lactate concentration (Boyar & Bowden, 1985; Milnes & Bowden, 1985) since this was the predominant fermentation product during growth in the chemostat at pH 4.1 (Table 4). This change in pattern could not be attributed to an increase in the numbers of bacteria with a homolactic fermentation pattern. It may be due to a switch in metabolism induced by prolonged growth at low pH or it may simply reflect the fact that glucose was no longer apparently limiting the growth of the community (Table 2) since lactate became the major fermentation product when similar mixed cultures were grown under glucose-excess conditions at pH 7.0 (Marsh *et al.*, 1983; McKee *et al.*, 1985). The high acid tolerance of *V. alcalescens* observed here might also account for the relatively large numbers of this species found in the stomach of patients with, for example, dyspepsia (Borriello *et al.*, 1985) or when the pH is raised to around pH 4.0 after drug treatment (Milton-Thompson *et al.*, 1982).

A number of other changes in the metabolism of bacteria were observed during growth at low pH in the chemostat. Washed cells produced more acid after a pulse of glucose, fructose or lactose than did the communities cultured at neutral pH, while amino acid metabolism was

markedly decreased. The latter may be of great significance in plaque ecology since base production from the metabolism of salivary peptides and amino acids is considered to be important in maintaining the integrity of enamel (Kleinberg *et al.*, 1976). When pH control was restored, the composition (with the exception of *B. intermedius*) and behaviour of the community rapidly reverted to that seen originally at pH 7.0. An inverse relationship in the proportions of *S. sanguis* and *S. mutans* was found in this study during growth at neutral and low pH, which was similar to that observed in human plaque (de Stoppelaar *et al.*, 1970). The findings *in vivo* were attributed to the availability of dietary carbohydrates. However, a consideration of the results acquired here and in a previous chemostat study (McKee *et al.*, 1985) suggests that the inverse relationship may be more of a reflection of the pH in plaque resulting from the catabolism of sugars rather than of the concentration of carbohydrate *per se*.

In conclusion, this study has confirmed the importance of the aciduricity (acid tolerance) of bacteria in dental plaque ecology. A lowering of the pH of plaque would suppress the numbers of several bacterial populations found at healthy sites while increasing the proportion of bacteria associated with the development of a carious lesion. This change in flora would decrease base production from the metabolism of salivary components, increase the rate of acid production (especially lactic acid) from pulses of dietary carbohydrates, and extend the pH range over which glycolysis can occur. While no attempt was made here to simulate the mouth directly, the results serve to highlight the potential of the chemostat in studies of dental plaque ecology. We plan to study the effect of other dietary components and antimicrobial agents on the composition and metabolism of these defined communities.

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