Environmental Regulation of Carbohydrate Metabolism by *Streptococcus sanguis* NCTC 7865 Grown in a Chemostat

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Carbohydrate metabolism by the oral bacterium *Streptococcus sanguis* NCTC 7865 was studied using cells grown in a chemostat at pH 7.0 under glucose or amino acid limitation (glucose excess) over a range of growth rates ($D = 0.05\text{ h}^{-1}$ – 0.4 h^{-1}). A mixed pattern of fermentation products was always produced although higher concentrations of lactate were formed under amino acid limitation. Analysis of culture filtrates showed that arginine was depleted from the medium under all conditions of growth; a further supplement of 10 mM-arginine was also consumed but did not affect cell yields, suggesting that it was not limiting growth. Except at the slowest growth rate ($D = 0.05\text{ h}^{-1}$) under glucose limitation, the activity of the glucose phosphotransferase (PTS) system was insufficient to account for the glucose consumed during growth, emphasizing the importance of an alternative method of hexose transport in the metabolism of oral streptococci. The PTS for a number of sugars was constitutive in *S. sanguis* NCTC 7865 and, even though the cells were grown in the presence of glucose, the activity of the sucrose-PTS was highest. The glycolytic activity of cells harvested from the chemostat was affected by the substrate, the pH of the environment, and their original conditions of growth. Glucose-limited cells produced more acid than those grown under conditions of glucose excess; at slow growth rates, in particular, greater activities were obtained with sucrose compared with glucose or fructose. Maximum rates of glycolytic activity were obtained at pH 8.0 (except for cells grown at $D = 0.4\text{ h}^{-1}$ where values were highest at pH 7.0), while slow-growing, amino acid-limited cells could not metabolize at pH 5.0. These results are discussed in terms of their possible significance in the ecology of dental plaque and the possible involvement of these bacteria in the initiation but not the clinical progression of a carious lesion.

INTRODUCTION

Streptococci can be readily isolated from all surfaces in the mouth and comprise a large proportion (approximately 28%) of the total cultivable flora of dental plaque (see Marsh & Martin, 1984). Oral streptococci can be separated into several distinct species, the numbers of which vary from site to site in both healthy and diseased mouths. For example, while *Streptococcus sanguis* is commonly present in relatively high numbers on healthy enamel surfaces, *Streptococcus mutans* is irregularly isolated and is normally only a minor component of dental plaque, except at sites which develop dental caries (for examples see Hardie *et al.*, 1977; Huis in t’Veld *et al.*, 1979; Loesche & Straffon, 1979).

The association between *S. mutans* and human dental caries has resulted in a considerable amount of research on the properties relating to the cariogenicity of this group of bacteria. Included among these properties are the ability to ferment rapidly to acid a number of dietary carbohydrates, the ability to tolerate and continue to metabolize sugars in the low pH

Abbreviations: PEP, phosphoenolpyruvate; PTS, phosphotransferase.

environments so produced, and the production of copious quantities of water-insoluble polysaccharides from sucrose which are involved in consolidating the attachment of cells to enamel and to one another, and which may contribute to the localization of acidic fermentation products (for reviews see Van Houte, 1980; Marsh & Martin, 1984). On the other hand, although *S. mutans* undoubtedly plays a major role in dental caries, lesions can sometimes develop in the absence of this species (Hardie *et al.*, 1977; Huis in t'Veld *et al.*, 1979; Loesche & Straffon, 1979). Furthermore, other oral bacteria can produce caries, albeit usually to a lesser extent than *S. mutans*, in experimental animals (Drucker & Green, 1981) but much less is known about the metabolism of these organisms. Consequently, we have undertaken a study of the growth and carbohydrate metabolism of a representative strain of *S. sanguis*, a species that can act as an opportunistic pathogen, for example when gaining entry to the blood stream following a tooth extraction, but which is not generally considered to play a major role in the aetiology of dental caries in man. Cells were grown in a chemostat under a range of environmental conditions to closely simulate their behaviour in the mouth. Results relating to the production of extracellular polysaccharides have been published previously (Keevil *et al.*, 1983, 1984*b*; West *et al.*, 1984).

METHODS

Bacterial strain and growth conditions. *Streptococcus sanguis* NCTC 7865 was grown under glucose (55.6 mM) or amino acid limitation (glucose excess, 166.7 mM) at 37 °C in a chemostat with a 550 ml working capacity using a modified (Marsh *et al.*, 1982), filter-sterilized, chemically defined medium (Carlsson, 1970). The pH was maintained automatically at 7.0 ± 0.1 by the addition of 2 M-NaOH, and the gas phase was 95% N₂ and 5% CO₂ (flow rate 160 ml min⁻¹). The cells were grown at dilution rates (*D*) of 0.05, 0.1, 0.2 and 0.4 h⁻¹, which correspond to mean generation times of 13.9, 6.9, 3.5 and 1.7 h, respectively. Each culture was allowed to reach equilibrium for at least 10 mean generation times before harvesting.

Cell preparation. 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 with 200 mM-KCl and centrifuged twice (8000 *g* for 10 min) and resuspended in 200 mM-KCl to give approximately 30 mg (dry wt) cells ml⁻¹, and were stored on ice until used.

Dry weight measurement. Known volumes of washed cells were filtered through pre-dried, pre-weighed polycarbonate membrane filters, pore size 0.4 µm (Sterilin). The cells on the filters were washed twice with sterile distilled water, and the filters were heated for 2 min to constant weight in a microwave oven. Estimations were done in duplicate and dry weights were calculated by differential weighing.

Glycolytic activity by washed cells. Washed cells were further diluted to give a final cell concentration of 5 mg (dry wt) ml⁻¹ in 5 ml 200 mM-KCl. Acid production by these cells from storage material (endogenous metabolism) and after a pulse of glucose, sucrose or fructose at 3 mM was measured at 37 °C under a constant flow of O₂-free N₂ in a pH-stat (Radiometer, Copenhagen, Denmark) as (1) a pH fall from pH 7.0, and (2) a rate at a constant pH (pH 5.0, 6.0, 7.0 and 8.0) using 0.1 M-KOH as the titrant. Rates of glycolytic activity were expressed as nmol acid neutralized min⁻¹ (mg cell dry wt)⁻¹ (Marsh *et al.*, 1982).

Assay for phosphoenolpyruvate-phosphotransferase (PEP-PTS) activity. Sugar transport via the PEP-PTS system was assayed with whole cells made permeable by adding 0.01 vol. toluene. The assay mixture and toluene treatment were as described by Hamilton & Ellwood (1978). Net activity was expressed as nmol pyruvate formed min⁻¹ (mg cell dry wt)⁻¹.

Rate of glucose transport in the chemostat. Glucose uptake by cells growing in each chemostat was calculated from the equation of Herbert & Kornberg (1976):

$$q_s = D (S_0 - S)/(x \times 60)$$

where q_s = rate of glucose utilization [nmol (mg cell dry wt)⁻¹], D = dilution rate (h⁻¹), S_0 = glucose concentration in the inflowing medium (nmol ml⁻¹), S = glucose concentration in the culture supernatant (nmol ml⁻¹) and x = community dry weight (mg ml⁻¹).

Rate of acid production in the chemostat. Because *S. sanguis* NCTC 7865 produced acidic fermentation products and because the chemostat cultures were maintained at pH 7.0 by the automatic addition of 2 M-NaOH, it was possible to determine the rate of acid production from the equation of Keevil *et al.* (1984*a*):

$$q_{\text{acid}} = AD_{\text{acid}}/60x$$

where q_{acid} = rate of alkali addition [nmol (mg cell dry wt)⁻¹], A = concentration of alkali (nmol ml⁻¹), x = community dry weight (mg ml⁻¹) and D_{acid} = dilution rate for acid (volume of alkali added per h per volume of culture vessel).

Analytical procedures. Amino acids in the uninoculated medium and the culture supernatant were analysed regularly as described previously, together with a measurement of the residual glucose and culture turbidity (Ellwood *et al.*, 1974). Culture filtrates were analysed for volatile and non-volatile acid products of fermentation by the methods of Hamilton & Ellwood (1978).

RESULTS

Growth parameters in the chemostat

At all growth rates, glucose could not be detected in the culture filtrates from the glucose-limited chemostat, while glucose was always present in supernatants from the amino acid-limited chemostat (Table 1). Arginine was depleted from the medium under both glucose-limited and glucose-excess conditions (Table 2). However, it was not limiting the growth of *S. sanguis* NCTC 7865 under glucose limitation since a further addition of 10 mM-arginine did not significantly increase the cell yield (Table 3). Molar growth yields were two- to threefold higher under glucose limitation compared to glucose excess conditions (Table 1). Although values are cited as $Y_{\text{molar glucose}}$, arginine could also have been acting as a carbon and energy source by providing carbamyl phosphate for substrate level phosphorylation. The increase in concentration of ornithine in culture supernatants after a 10 mM pulse of arginine provided some experimental support for this proposal (Table 3).

A mixed pattern of fermentation products was formed under all conditions of growth (Table 1). The concentration of lactate in culture filtrates together with the percentage of glucose carbon recovered as fermentation products increased with the dilution rate under glucose limitation, while the highest concentration of lactate was formed at the slowest growth rate under glucose excess conditions. Under all conditions of growth, the percentage of carbon recovered as fermentation products was less than 100% (Table 1), suggesting that considerable quantities of storage material were being produced.

The rates of glucose consumption and acid production increased with the dilution rate under glucose and amino acid limitation (Fig. 1). In contrast, the activity of the PEP-mediated glucose-PTS remained constant or decreased slightly as the growth rate increased. Except at the slowest rate of growth ($D = 0.05 \text{ h}^{-1}$) under glucose limitation, the activity of the PTS was not commensurate with the measured rate of glucose utilization in the chemostat (Fig. 1).

Glycolytic and PTS activity of washed cells

The response of cells harvested from the chemostat to a pulse of carbohydrate was measured in terms of the activity of their PEP-PTS transport systems and their ability to convert these sugars to acidic fermentation products. A comparison was also made of the glycolytic activity of cells metabolizing polymers stored intracellularly during growth (endogenous metabolism). Glycolytic activity was measured as the terminal pH after 15 min metabolism (Table 4) and as a rate at a constant pH (Table 5, Fig. 2). Significant endogenous metabolism was measured in all cells irrespective of the conditions of growth but, perhaps surprisingly, some of the highest activities were found with cells that had been grown under glucose limitation (Tables 4 and 5). Cells grown under glucose limitation always produced more acid from a pulse of glucose than those grown under amino acid limitation but this relationship did not hold for sucrose and fructose. Indeed, the lowest terminal pH value and the fastest rate of acid production were obtained from amino acid-limited cells grown at $D = 0.05 \text{ h}^{-1}$ and metabolizing sucrose (Tables 4 and 5). In general, fructose was metabolized more slowly than the other two sugars. These results reflected the measured activities of the PTS under different growth conditions. Growth rate had little effect on the glucose-PTS above $D = 0.1 \text{ h}^{-1}$ (Fig. 1) but the activity of the glucose-, sucrose- and fructose-PTS was greater in cells grown under carbohydrate limitation. At $D = 0.1 \text{ h}^{-1}$, in spite of the cells being grown on glucose, the activity of the sucrose-PTS was approximately twice that for glucose; activities for the glucose-, sucrose- and fructose-PTS were 22.5, 55.0 and 9.8 nmol (mg cell dry wt) $^{-1} \text{ min}^{-1}$, respectively, under glucose limitation and 13.4, 25.5 and 4.0 nmol (mg cell dry wt) $^{-1} \text{ min}^{-1}$, respectively, under glucose excess conditions.

Table 1. *Yields and fermentation products of S. sanguis NCTC 7865 grown in a chemostat*Cells were grown at pH 7.0 under glucose or amino acid limitation (glucose excess) at different dilution (growth) rates (*D*).

<i>D</i> (h ⁻¹)	Nutrient limitation	Yield (mg dry wt ml ⁻¹)	Residual glucose (mg ml ⁻¹)	$Y_{\text{molar glucose}}$ [g dry wt cells (mol glucose consumed) ⁻¹]	Fermentation products (mm)				Percentage of glucose carbon recovered as fermentation products
					Ethanol	Acetate	Formate	Lactate	
0.05	Glucose	0.92	0	16.2	34.1	38.3	38.0	10.6	64.4
	Amino acid	0.61	12.3	9.0	31.1	6.3	26.1	63.1	86.4
0.1	Glucose	1.25	0	23.4	44.4	23.0	23.5	9.3	55.9
	Amino acid	0.66	12.0	9.9	41.5	16.7	60.0	20.1	70.4
0.2	Glucose	1.67	0	30.7	37.8	31.1	41.3	43.3	92.8
	Amino acid	0.67	10.7	10.3	18.7	20.5	53.7	25.5	54.4
0.4	Glucose	1.36	0	25.2	39.1	31.6	41.3	45.5	95.9
	Amino acid	0.61	13.9	13.2	22.0	17.2	43.2	21.9	67.3

Table 2. *Amino acid metabolism by S. sanguis NCTC 7865*

Cells were grown in a chemostat at pH 7.0 under glucose or amino acid limitation (glucose excess) at different dilution (growth) rates (*D*) in a chemically defined medium. Metabolism is expressed as a percentage increase or decrease in the concentration of an amino acid in culture supernatants compared with the uninoculated medium.

Amino acid	Concn (mM) in uninoculated medium		<i>D</i> (h ⁻¹) . . .	Percentage increase (+) or decrease (-)							
	Glucose limitation	Amino acid limitation		Glucose limitation				Amino acid limitation			
				0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4
Alanine	1.20	0.44		+10	+14	+87	+48	+33	+10	-27	+53
Arginine	0.45	0.16		-100	-100	-100	-100	-100	-100	-100	-100
Aspartic acid	0.30	1.20		-39	-31	-53	-30	-49	-34	-55	-41
Cystine	1.24	0.36		-25	-1	-1	-15	-50	0	+6	-25
Glutamic acid	3.23	1.24		0	-4	-15	0	-59	0	-46	-38
Glycine	2.60	0.95		-50	-11	-18	-16	-43	-7	-39	-27
Histidine	0.46	0.18		-86	-77	-62	-86	-80	-79	-46	-82
Isoleucine	1.40	0.57		-40	-34	-29	-48	-81	-64	-80	-20
Leucine	1.73	0.61		-34	-44	-32	-23	-80	-68	-80	-32
Lysine	1.60	0.43		-36	-50	-40	-44	-64	-53	-82	-43
Methionine	1.10	0.40		-23	-9	-21	-18	-41	+17	-33	-9
Ornithine	tr	tr		*	*	*	*	*	*	*	*
Phenylalanine	0.62	0.22		-31	-40	-32	-29	-63	-47	-47	-25
Proline	0.90	0.31		-37	-2	-2	-4	-25	-5	+14	0
Serine	1.00	0.36		-56	-77	-67	-50	-88	-92	-88	-74
Threonine	0.87	0.31		-57	-61	-44	-44	-90	-85	-84	-37
Tryptophan	0.77	0.30		-25	0	-17	-8	-70	-20	-20	-36
Tyrosine	0.58	0.20		-42	-40	-33	-36	-77	-47	-38	-22
Valine	0.89	0.30		-45	-46	-39	-31	-70	-54	-53	+6

tr, Trace.

* Amino acid detected in culture filtrates but not in the uninoculated medium.

Fig. 1. A comparison of the rates of glucose consumption (q_s , $\circ$) and acid production (q_{acid} , $\blacksquare$) by *S. sanguis* NCTC 7865 grown in a chemostat at different growth rates under (a) glucose limitation or (b) amino acid limitation. The activity of the glucose-PTS ($\blacktriangle$) is also shown. The activity was measured in cells harvested from the chemostat as described in the text. All rates of activity are expressed as nmol glucose, acid or pyruvate formed (mg cell dry wt)⁻¹ min⁻¹.

Table 3. *Effect on cell yields of supplementing a glucose-limited culture of S. sanguis NCTC 7865 with arginine*

Cells were grown in a chemically defined medium (M3) at pH 7.0 and at a dilution rate of 0.1 h⁻¹.

Growth medium	Arginine (mM) in:		Ornithine (mM) in:		OD ₅₄₀ of culture*	Cell yield* (mg dry wt ml ⁻¹)
	Medium	Culture filtrate	Medium	Culture filtrate		
M3	0.45	0	0.02	0.28	3.20 ± 0.29 (7)	1.20
M3 + 10 mM-arginine	10.45	0	0.02	3.20	3.40 ± 0.29 (9)	1.22 ± 0.01 (2)

* Results are expressed as mean ± SD with the number of experiments in parentheses.

Table 4. *Acid production by S. sanguis NCTC 7865*

Cells were grown in a chemostat at pH 7.0 under glucose or amino acid limitation (glucose excess) at different dilution rates (D). Cells were harvested by centrifugation, and were washed and resuspended in 200 mM-KCl. Acid production was measured as a pH fall from pH 7.0 after 15 min endogenous metabolism of storage material, or after a pulse of excess glucose, sucrose or fructose. The number of determinations using fresh cells prepared on different days is given in parentheses.

D (h ⁻¹)	Nutrient limitation	Terminal pH (mean ± SD)			
		Endogenous	Glucose	Sucrose	Fructose
0.05	Glucose	5.61 ± 0.18 (4)	4.87 ± 0.07 (5)	4.81 ± 0.03 (4)	5.04 ± 0.11 (3)
	Amino acid	6.10 ± 0.06 (3)	5.64 ± 0.28 (3)	4.27 ± 0.25 (3)	4.89 ± 0.30 (3)
0.1	Glucose	5.94 ± 0.04 (3)	4.54 ± 0.09 (4)	4.58 ± 0.08 (3)	4.80 ± 0.06 (3)
	Amino acid	5.13 ± 0.20 (7)	5.00 ± 0.19 (9)	4.72 ± 0.08 (5)	4.93 ± 0.10 (4)
0.2	Glucose	5.20 ± 0.26 (3)	4.49 ± 0.15 (3)	4.65 ± 0.15 (3)	4.70 ± 0.05 (3)
	Amino acid	6.00 ± 0.09 (4)	4.66 ± 0.11 (4)	4.74 ± 0.11 (4)	5.26 ± 0.21 (4)
0.4	Glucose	5.47 ± 0.23 (3)	4.63 ± 0.10 (3)	4.82 ± 0.08 (3)	4.88 ± 0.11 (3)
	Amino acid	6.30 ± 0.28 (4)	4.73 ± 0.08 (4)	4.57 ± 0.03 (3)	4.88 ± 0.18 (4)

Fig. 2. Glycolytic activity of KCl-washed cells of *S. sanguis* NCTC 7865 harvested from a chemostat at different dilution rates ($\diamond$, $\blacklozenge$, 0.05 h^{-1} ; $\circ$, $\bullet$, 0.1 h^{-1} ; $\triangle$, $\blacktriangle$, 0.2 h^{-1} ; $\square$, $\blacksquare$, 0.4 h^{-1}), and measured in a pH-stat at different pH values. Cells were grown under (a) glucose limitation or (b) amino acid limitation. Rates of glycolytic activity are expressed as $\text{nmol acid neutralized (mg cell dry wt)}^{-1}\text{ min}^{-1}$.

Table 5. Glycolytic activity of *S. sanguis* NCTC 7865

Cells were grown in a chemostat at pH 7.0 under glucose or amino acid limitation (glucose excess) at different dilution rates (D). Cells were harvested by centrifugation, and were washed and resuspended in 200 mM-KCl. Glycolytic activity was measured as a rate at pH 7.0 from the endogenous metabolism of storage material, or after a pulse of excess glucose, sucrose or fructose. Units of glycolytic activity are $\text{nmol acid neutralized at pH 7.0 (mg cell dry wt)}^{-1}\text{ min}^{-1}$. The number of determinations using fresh cells prepared on different days is given in parentheses.

D (h^{-1})	Nutrient limitation	Glycolytic activity (mean $\pm$ SD)			
		Endogenous substrate	Glucose	Sucrose	Fructose
0.05	Glucose	14 ± 4 (6)	40 ± 10 (6)	31 ± 11 (5)	22 ± 9 (5)
	Amino acid	20 ± 2 (6)	19 ± 3 (7)	69 ± 6 (2)	39 ± 10 (5)
0.1	Glucose	9 ± 3 (5)	57 ± 9 (5)	50 ± 12 (3)	35 ± 6 (2)
	Amino acid	19 ± 1 (2)	21 ± 5 (8)	23 ± 1 (4)	19 ± 4 (2)
0.2	Glucose	16 ± 3 (4)	49 ± 7 (4)	53 ± 10 (4)	37 ± 7 (4)
	Amino acid	10 ± 4 (3)	40 ± 6 (5)	40 ± 6 (3)	22 ± 1 (3)
0.4	Glucose	23 ± 4 (3)	45 ± 13 (3)	36	17
	Amino acid	10 ± 2 (5)	41 ± 9 (5)	41 ± 1 (2)	24 ± 3 (2)

Although the cells were grown in the chemostat at pH 7.0, the maximum glycolytic rates by washed cells were at pH 8.0 except for those grown at $D = 0.4\text{ h}^{-1}$ (Fig. 2). At every pH tested, cells grown under glucose limitation produced acid at a faster rate than cells grown under amino acid limitation. Also, glucose-limited cells were more aciduric, i.e. they could continue to metabolize at lower external pH values, than those grown under amino acid limitation (Fig. 2). Indeed, only the faster-growing cells ($D = 0.2$ and 0.4 h^{-1}) from the amino acid-limited chemostat could metabolize glucose at pH 5.0.

DISCUSSION

Until recently, the PEP-PTS was believed to be the sole sugar transport system in oral streptococci. However, in the present study, it was clear that, except at $D = 0.05\text{ h}^{-1}$ under glucose limitation, the activity of the PTS was insufficient to account for the concentration of

glucose being transported by *S. sanguis* during growth. Similar results have been reported for *S. mutans* catabolizing glucose (Ellwood *et al.*, 1979) and sucrose (Ellwood & Hamilton, 1982), and for *Actinomyces viscosus* growing on glucose (Hamilton & Ellwood, 1983). Recent studies, using a PTS-defective mutant of *S. mutans* (Hamilton & St Martin, 1982) and a range of streptococci in which the PTS was inhibited by chlorhexidine (Keevil *et al.*, 1984c; Marsh *et al.*, 1984), have shown that the residual, non-PTS attributable glycolytic activity could be completely abolished by protonophores such as gramicidin and by the ATP synthetase inhibitor *N,N'*-dicyclohexylcarbodiimide, suggesting that the additional hexose transport was driven by the energized membrane. The finding that valinomycin had less of an inhibitory effect than gramicidin on both glycolysis (Keevil *et al.*, 1984c) and growth (West *et al.*, 1984) of *S. sanguis* suggested that ΔpH rather than $\Delta\psi$ was the major protonmotive force component responsible for driving glucose transport. The possession of two independent sugar transport systems (PTS and protonmotive-force driven) would enable oral streptococci to respond efficiently to the oscillating pH and nutrient conditions (particularly in terms of carbohydrate concentration) that prevail in the mouth.

A surprising feature of our study was the significant levels of endogenous metabolism exhibited by cells grown under glucose limitation since, in most bacteria, storage polysaccharides are synthesized only during periods of carbohydrate excess. However, in *S. sanguis* NCTC 7865, a novel reaction has been described whereby glucose can be phosphorylated by acetyl phosphate or carbamyl phosphate directly to glucose 1-phosphate, the precursor of intracellular storage material (Keevil *et al.*, 1984a). The depletion of arginine and the production of acetic acid would have ensured a continual supply of both phosphoryl donors under all growth conditions. The involvement of acetyl phosphate and carbamyl phosphate in substrate level phosphorylation might also account, in part, for the higher than theoretically predicted cell yields of *S. sanguis* NCTC 7865 during glucose-limited growth (Keevil *et al.*, 1984c). The ability of an organism to synthesize storage material over a range of environmental conditions might also aid its survival in dental plaque since it has been suggested that polysaccharides help to preserve cell integrity and viability during conditions of nutrient starvation, and they can also be used as an energy source in the absence of exogenous carbohydrates (Dawes, 1976). Furthermore, this property may be important in disease since the production of acid by endogenous metabolism in the absence of dietary carbohydrates has been proposed as an important characteristic of cariogenic bacteria (Tanzer *et al.*, 1976).

The pattern of amino acid utilization by *S. sanguis* NCTC 7865 also differed from that reported for other oral bacteria. In the present study, arginine was depleted under all conditions of growth, whereas cysteine and asparagine, respectively, were the predominant amino acids consumed by *S. mutans* (Hamilton & Ellwood, 1978) and *A. viscosus* (Hamilton & Ellwood, 1983). Mixed substrate utilization is a common feature of bacterial communities so that the observed preferences for different amino acids might enable these organisms to avoid competition and co-exist under carbohydrate-limited conditions, as has been observed both in mixed continuous cultures (Marsh *et al.*, 1983; Kemp *et al.*, 1983; McKee *et al.*, 1984) and in dental plaque itself.

Although it is now generally accepted that no single organism is the sole pathogen in dental caries, certain bacteria, in particular *S. mutans* and *Lactobacillus* spp. (Hardie *et al.*, 1977; Loesche & Straffon, 1979), have been identified as having a greater role in the disease process than species such as *S. sanguis*. When comparing cariogenic with non-cariogenic bacteria, properties such as the rate of carbohydrate transport and acid production in response to a pulse of sugar are as important as the terminal pH reached (enamel demineralization accelerates below pH 5.5). Furthermore, the type of acid is important in the development of carious lesions *in vitro*, acetic acid being preferentially adsorbed by enamel (Featherstone *et al.*, 1981; Geddes *et al.*, 1984). The data presented here show that *S. sanguis* NCTC 7865 produces more acetic acid and has a greater endogenous metabolism under certain conditions of growth than does *S. mutans*. The PTS transport system for a number of sugars was constitutive in *S. sanguis* and, unlike the system in *S. mutans* (St Martin & Wittenberger, 1979; Slee & Tanzer, 1980), its activity was less affected by growth conditions. Moreover, under certain environmental

conditions, the activity of the sucrose-PTS of *S. sanguis* was greater and the terminal pH from sucrose lower than the values obtained with *S. mutans* (Ellwood *et al.*, 1979; Hamilton & Ellwood, 1978). Paradoxically, however, the rate of acid production from any sugar was always much slower than the values obtained with *S. mutans*. Another major difference was the inability of *S. sanguis* to produce acid at low pH values, which is in agreement with recent work by Harper & Loesche (1984). These findings suggest that *S. sanguis* might have sufficient glycolytic activity to contribute to the early stages of dental caries, although its apparent lack of acid tolerance makes it a less likely candidate for being involved in the clinical progression of a lesion through the enamel to the underlying tissues.

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