Effect of pH Upon Sucrose and Glucose Catabolism by the Various Genogroups of *Streptococcus mutans*

D. S. HARPER* and W. J. LOESCHE

Schools of Dentistry and Medicine, The University of Michigan, Ann Arbor, Michigan 48109

Sucrose and glucose catabolism by seven strains of Streptococcus mutans *belonging to six serotypes was assayed at pH's 6.5, 5.0, 4.5, and 4.0 with a radioisotopic tracer assay. The strains differed in their patterns of metabolic stimulation and inhibition at the different pH levels, falling into groups corresponding to the genetic groups described by Coykendall. The genogroup I (serotypes* c *and* e*) strains were the most acid-tolerant, having a pH optimum for lactic acid production at pH 5.0. These data furnish additional metabolic confirmation of the distinctiveness of these* S. mutans *subgroups.*

J Dent Res 62(5):526-531, May 1983

Introduction.

Streptococcus mutans is involved in the initiation and progression of carious lesions,[1-3] especially in fissure and interproximal sites.[4] Along with the ability to produce adherent extracellular polysaccharides (ECP)[1,5] and energy reserves of intracellular polysaccharides (ICP),[6] aciduricity (acid tolerance) is probably very closely related to the high cariogenicity of *S. mutans*. Harper and Loesche (manuscript in preparation) have shown that *S. mutans* Ingbritt, a serotype *c* strain, is more aciduric at pH levels less than or equal to pH 5.0 than are strains of *Streptococcus sanguis* or *Actinomyces viscosus*, which are less cariogenic than *S. mutans*.[1,7]

S. mutans consists of groups of phenotypically similar organisms which differ in terms of their serologic,[8] genetic,[9] and metabolic[8,10] characteristics. Coykendall[9] has proposed the recognition of four and possibly five species of organisms among the *S. mutans* group. The functional significance of these differences is not currently known, although the various groups are distributed differently in human populations,[11,12] and may have different cariogenic activities in animal models.[13]

The ability to ferment soluble carbohydrates at low pH levels may be an important determinant of the cariogenic potential of *S. mutans*. A previous investigation had shown that resting cell suspensions of a serotype *c* strain of *S. mutans* incubated, at pH 7.0, converted sucrose to acids and a variety of polysaccharides.[14] About 80% of the acids were identified as lactic acid, and about 50% of the polysaccharides appeared to be of the intracellular type (ICP). The measurements of lactic acid and ICP were technically easier than were the measurements of ECP and volatile acids.[15] Since lactate and ICP accounted for about 60% of the consumed sucrose, they represented major metabolic pathways for *S. mutans* under the conditions of the resting cell suspension.

In the present investigation, the production of lactic acid was determined in resting cell suspensions of seven *S. mutans* strains representing the four genetic groups defined by Coykendall.[9] The substrates included either glucose or sucrose, and their metabolism was assayed at pH levels ranging from 6.5 to 4.0. In this manner, it was possible to compare the ability of the genetic groups to metabolize either substrate under a range of pH levels that could be expected to occur in dental plaque.

The results showed that the genetic groups differed in their patterns of sucrose and glucose metabolism. Also, strains belonging to genetic group I (serotype *c*) exhibited a pH optimum at about pH 5.0.

Materials and methods.

Organisms. – The following strains of *Streptococcus mutans* (followed by their serotype and genetic group) were used in this study: Ingbritt (*c*/I), S (*c*/I), LM7 (*e*/I), OMZ 176 (*d*/III), 6715 (*g*/III), E49 (*a*/IV), and FAI (*b*/II). All strains were stock cultures of extensively studied *S. mutans* isolates with the exception of strain S, which was a recent human isolate. The strains were maintained as frozen blood suspensions on glass beads.[16] Biotypes of all strains were confirmed using the fermentation assay of Shklair and Keene.[10]

Carbohydrate metabolism assay. – Sucrose and glucose metabolism by resting cell suspensions of the various strains was assayed at pH 6.5, 5.0, 4.5, and 4.0, using an adaptation of the radio-isotopic tracer assay of Minah and Loesche.[14,15] Cultures were inoculated directly from glass bead stocks into 5 ml of Trypticase Soy Broth (TSB) and were incubated at 35° in an anaerobic atmosphere of 85% N_2, 10% CO_2, and 5% H_2 for from 16 to 18 h. No additional sucrose was added to the TSB, since it contains sufficient trace sucrose to induce sucrose-stimulated enzyme systems,[1] and additional sucrose would have led to increased cell aggregation.

Cultures were centrifuged, washed once, and re-suspended in from 3-5 ml Reduced Transport Fluid (RTF[17]), with care being taken to make all suspensions approximately equal in cell concentration, *i.e.*, about 10^8 CFU per ml. The cell suspensions were transported into an anaerobic chamber kept at 35°[18] and were dispersed by 20 s of mild sonication.§ Fifteen-μl aliquots were removed and were diluted in RTF for later determination of viable counts. To start the assay, 100 μl of the cell suspensions were added to 150 μl of RTF in one-half-dram glass vials containing 1μCi of U-^{14}C sucrose or glucose‡ (2 x 10^6 CPM/Ci). Additional cold sucrose or glucose had been added to bring the final concentration of the sugar in the reaction mixture to 0.1%.

The RTF buffers used for washing and suspending the cells were adjusted to pH 6.5, 5.0, 4.5, or 4.0 by mixing appropriate proportions of acidic and basic RTF stocks

Received for publication July 29, 1981
Accepted for publication August 10, 1982

*Present address: American Dental Association Health Foundation, Research Institute, 211 East Chicago Avenue, Chicago, IL 60611

Based on a dissertation submitted to the Graduate School, The University of Michigan, in partial fulfillment of the requirements for the Ph.D. degree

This investigation was supported in part by Public Health Services Grants DE-03423 and DE-03011 from the National Institute of Dental Research.

§ Kontes Glass Co. Cell Disruptor
‡ New England Nuclear

containing 9.96 mM lactic acid and 5.17 mM K_2HPO_4, respectively. At two, five, ten, 20, and 30 min, 1-μl aliquots of the assay suspensions were spotted on thin-layer chromatography sheets, following which the sheets were dried and developed in a solution of 2-butanone:tert butanol:formic acid:water (30:40:10:20).[15] Spots representing lactic acid and sugar were removed, and radioactivity was determined with a liquid scintillation counter. Approximately from 3 to 10% of total radioactivity was recoverable as lactic acid in actively metabolizing cultures, 0.5-2% as soluble polysaccharide, and 0.2-1% as insoluble polysaccharide. The majority of counts remained in the sucrose (when present), glucose, and fructose spots, and so, it can be assumed that the cells were always in sugar excess. Previous studies by Minah and Loesche[14,15] showed that resting cells of either plaque or *S. mutans* serotype *c* incubated with 0.1% sucrose at pH 7 produced five times more lactic acid than did the combined volatile acids: acetic, butyric, or propionic. Since the *S. mutans* strains used in this study also produced lactate as the major acid end product – *i.e.*, from 60 to 80% of the total acids could be accounted for as lactate – the other acidic end products were not quantified.

At the conclusion of the experiment, all reaction mixtures were immediately checked for terminal pH using a miniature glass pH electrode,¶ and were then frozen and stored until total protein content of each reaction mixture could be assayed by the microfluorometric technique of Undenfriend *et al.*[19] Terminal pH of the reaction suspensions was always within ± 0.3 pH units of the starting pH.

¶ Microelectrodes, Inc.

Data management and statistics. – Data obtained from the resting cell metabolism assays were processed using a Fortran-based computer program. Processed data were written into computer files and were analyzed using programs in the Michigan Interactive Data Analysis System (MIDAS). There was a considerable amount of non-significant, apparently random variation within strains, especially at low pH levels. Significant differences among various groups were determined using the non-parametric Mann-Whitney *U* test and are noted in the Tables.

Results.

Lactic acid production. – Lactic acid production from 0.1% sucrose and glucose was determined in resting cell suspensions of the different *S. mutans* strains at pH 6.5, 5.0, 4.5, and 4.0. Strains belonging to the same genetic groups exhibited similar patterns of stimulation and inhibition of acid production at the decreasing pH levels and frequently produced similar amounts of acid.

The results were normalized to both the CFU and the μg protein present per ml of cell suspension. The findings, both in direction and in magnitude, were similar in each case. For simplicity of presentation, only the results computed as ηM lactic acid formed per μg protein are displayed.

Resting cell suspensions of the genogroup I strains Ingbritt, S, and LM7 (Table 1) had pH optima at 5.0, in that they produced approximately from 160 to 200% more lactic acid from glucose at pH 5.0 than at pH 6.5. A similar pH optimum was seen in acid production from sucrose by

TABLE 1
EFFECT OF DECREASING pH ON LACTIC ACID PRODUCTION FROM 0.1% SUCROSE OR GLUCOSE BY *STREPTOCOCCUS MUTANS* GENOGROUP I STRAINS INGBRITT, S, AND LM7

Strain	Sugar	pH	ηM Lactic Acid per 0.2 μg Protein				
			2 min	5 min	10 min	20 min	30 min
Ingbritt	sucrose	6.5	3.40	4.88	5.12	5.64	6.52
Ingbritt	sucrose	5.0	2.80	5.60	7.72	11.64+	15.68+
Ingbritt	sucrose	4.5	2.26	2.36*	3.88*	5.84	8.80
Ingbritt	sucrose	4.0	1.84*	2.24	2.92	4.24*	5.92
Ingbritt	glucose	6.5	3.56	5.00	6.04	7.12	7.12
Ingbritt	glucose	5.0	4.18	5.94	8.16+	13.24+	17.70+
Ingbritt	glucose	4.5	3.10*	4.64	6.56	9.90	13.94+
Ingbritt	glucose	4.0	2.20	2.66*	3.84	8.78	7.44
S	sucrose	6.5	11.04	7.44	8.16	8.80	10.24
S	sucrose	5.0	13.16	17.28+	14.04+	19.32+	25.12+
S	sucrose	4.5	9.20	3.44	5.96	9.00	12.08
S	sucrose	4.0	0.40	0.64	1.04*	1.32	1.44*
S	glucose	6.5	6.10	7.98	8.72	9.60	10.40
S	glucose	5.0	17.14+	11.22+	17.62+	23.90+	29.84+
S	glucose	4.5	15.76+	18.74+	10.46+	13.30+	17.62+
S	glucose	4.0	7.48	8.04	5.08	2.70*	6.04
LM7	sucrose	6.5	7.12	7.44	6.08	9.40	10.52
LM7	sucrose	5.0	4.28	5.56	7.36	7.28	13.36
LM7	sucrose	4.5	2.96	4.44	6.60	9.36	13.08
LM7	sucrose	4.0	3.68	3.00	3.00	3.00	4.64
LM7	glucose	6.5	4.04	3.70	3.86	4.98	5.78
LM7	glucose	5.0	13.90+	16.36+	18.80+	23.86+	31.10+
LM7	glucose	4.5	3.32	4.72	6.46	10.56	11.94
LM7	glucose	4.0	2.14	4.48	4.16	7.16	8.70

+Significantly higher than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).
*Significantly lower than pH 6.5 ($P > 0.05$, Mann-Whitney *U* Test).
†Values represent mean of six determinations.

strains Ingbritt and S, but not by strain LM7. Acid production at pH 4.5, especially from glucose, was moderately stimulated in comparison to that at pH 6.5. There was some variability of acid production over time at pH 4.0, but this was not statistically significant. The amount produced at pH 4.0 was similar to that which was found at pH 6.5 except in Strain S, which tended to form less acid at the low pH (Table 1).

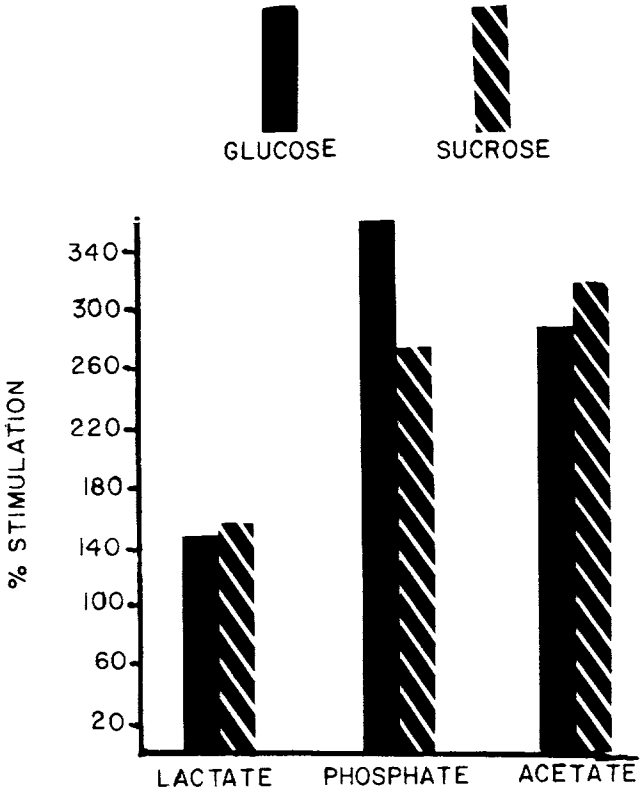

Fig. – Percent stimulation of lactic acid production from 0.1% glucose or sucrose at pH 5.0 (relative to pH 6.5) by resting cell suspensions of *S. mutans* Ingbritt incubated in three buffer systems. % stimulation = acid produced at pH 5.0 ÷ acid produced at pH 6.5 X 100. Stimulation, measured at 30 min, was statistically significant ($P < 0.05$, Mann-Whitney *U* Test) in all groups.

Resting cells of strain Ingbritt incubated at pH 6.5 and 5.0 in acetic acid-based and phosphoric acid-based buffers also demonstrated a stimulation of acid production at pH 5.0 relative to that at pH 6.5 (Fig.). This indicates that the increase in acid production at pH 5.0 was related to pH and not lactate ion concentration.

Genogroup III strains, OMZ 176 and 6715 (Table 2), produced acid from both sucrose and glucose at similar rates at pH 6.5 and 5.0. Acid production was inhibited approximately 30 to 50% at pH 4.5 and 70 to 90% at pH 4.0. The amount of acid produced by the genogroup III strains was greater than or equal to that of the genogroup I strains at pH 6.5, equal to or slightly less than I at pH 5.0 and 4.5, and significantly less than the genogroup I strains at pH 4.0.

The genogroup IV strain, E49, produced appreciably less acid at the acidic pH levels than did the strains from other genogroups (Table 3). Acid production at pH 6.5 was comparable to that of the genogroup I strains. Significantly more acid was formed from glucose at pH 5.0 relative to pH 6.5, while acid production from sucrose at the two pH levels was similar. Acid production at pH 4.5 and 4.0 was almost completely inhibited. Unlike the genogroup I and III strains, the viable recovery of E49 cells was significantly lower at pH 4.5 and 4.0 than at pH 6.5 or 5.0. Thus, cell death at low pH's may partially account for the substantial inhibition of acid production at these acidic pH levels.

The genogroup II strain FA1 also showed decreasing rates of acid production at the acidic pH levels (Table 4). A clear difference between the sugars was observed, in that lactic acid production from glucose, but not sucrose, was maintained at pH 5.0. Acid production from both sugars was significantly inhibited at pH 4.5 and 4.0, although the inhibition of the glucose-incubated cells was less severe than that of the sucrose-incubated cells. Recovery of viable cells was reduced at pH 4.5 and 4.0, although the reduction was not statistically significant.

TABLE 2
EFFECT OF DECREASING pH ON LACTIC ACID PRODUCTION FROM 0.1% SUCROSE OR GLUCOSE BY *STREPTOCOCCUS MUTANS* GENOGROUP III STRAINS OMZ 176 AND 6715

			ηM Lactic Acid per 0.2 μg Protein				
Strain	Sugar	pH	2 min	5 min	10 min	20 min	30 min
OMZ 176	sucrose	6.5	4.84	6.48	7.32	10.04	11.68
OMZ 176	sucrose	5.0	3.56	5.12	7.04	10.72	11.76
OMZ 176	sucrose	4.5	4.40	3.44*	4.84*	6.88*	7.88*
OMZ 176	sucrose	4.0	0.32*	0.72*	1.12*	1.20*	1.88*
OMZ 176	glucose	6.5	4.80	5.54	7.14	10.42	12.34
OMZ 176	glucose	5.0	7.56	4.36	7.12	10.00	11.22
OMZ 176	glucose	4.5	1.88*	2.50*	3.86*	6.38*	7.90*
OMZ 176	glucose	4.0	0.80*	1.40*	1.60*	2.48*	2.70*
6715	sucrose	6.5	5.52	7.60	9.20	12.16	12.84
6715	sucrose	5.0	5.76	8.00	9.20	13.80	16.48+
6715	sucrose	4.5	2.00*	2.36*	2.96*	5.28*	6.56*
6715	sucrose	4.0	0.48*	0.48*	0.72*	0.60*	0.72*
6715	glucose	6.5	8.68	12.94	14.80	16.60	19.88
6715	glucose	5.0	6.78	10.40	15.10	19.50	22.96
6715	glucose	4.5	2.82*	3.60*	6.30*	9.60*	12.88
6715	glucose	4.0	0.06*	0.44*	0.70*	0.86*	1.78*

+Significantly higher than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).
*Significantly lower than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).

Discussion.

Although the different genetic, serologic, and metabolic groups of *S. mutans* have been well-studied, the functional significance of these groupings as far as the pathogenesis of human dental decay is not yet well understood. The observation in this study – that strains belonging to the same genetic group, but not necessarily the same serotype of *S. mutans*, exhibited similar patterns of lactic acid production at low pH levels – indicates that these genetic groupings correlate well with the aciduricity of these strains, and lends further support for the taxonomic division proposed by Coykendall.[9]

Genogroup I strains showed a clear pH optimum of 5.0 compared to the genogroups II and IV strains. The genogroups I and III strains were more metabolically-active at pH's 4.5 and 4.0 than were the genogroups II and IV strains. The genogroup I strains were the more aciduric of the strains tested, whereas the genogroup IV strain was the least aciduric. Glucose was usually more rapidly converted to lactic acid than was sucrose, presumably because fewer molecules were diverted to extracellular polysaccharides.

The impact of these differences in aciduricity upon the cariogenicity of the various genogroups is not known. However, the genogroup I strains, which were the most aciduric, are the most commonly isolated human strains of *S. mutans*.[11,12] Genotype III strains, which are the second most frequently encountered human isolates of *S. mutans*, were also highly active at pH 5.0. This suggests that genogroups I and III strains could be selected for by the acid environment present at the plaque-enamel interface where enamel demineralization occurs *in vivo*. This phenomenon could explain the significantly higher levels of these *S. mutans* genotypes which are found in pits and fissures during caries initiation,[3] and later when cavitation is present.[1,2]

Strain S was the most aciduric genogroup I isolate tested, producing significantly more lactic acid at pH 4.5 from glucose than did the other strains (Table 1). In separate investigations, strain S was the only *S. mutans* isolate that could be reliably established in an artificial fissure model[20] in the mouths of human volunteers using a mouth rinse or pre-incubated fissures. The present findings of this organism's marked aciduricity suggest that aciduricity may be a factor in the establishment of various *S. mutans* genotypes in a retentive site such as a fissure. In this regard, genogroups II and IV strains, which were considerably less aciduric than the genogroups I and III strains, did not establish in the mouths of human subjects, even when the artificial fissure model was used.[20]

While the stimulation of intracellular polysaccharide production at decreasing pH has been described,[21] enhanced acid production from both glucose and sucrose at pH 5.0 by *S. mutans* strains belonging to genogroup I has not been previously reported. Hamilton and Elwood[22] reported that *S. mutans* cells of strain Ingbritt grown at a constant pH of 5.5 in a chemostat had more glycolytic activity than did cells grown at a constant pH of 6.5 or 6.0. They did not find an increase in glycolytic activity in resting cell suspensions incubated at pH 5.5 in comparison to cells incubated at pH 6.5. However, they did not evaluate metabolism at pH levels lower than 5.5. The present studies report results obtained in batch-grown cultures. Since the majority of bacterial cells in plaque appears to be either very slow-growing or stationary,[23] the use of cells from the early stationary phase, as was done in this study, might be expected to reflect the metabolic response of plaque bacteria. However, because metabolic activities of batch-grown cells may differ significantly from those of continuously cultured cells,[23] it would be very useful to carry out studies similar to those done here using continuously cultured cells.

The use of relatively low sucrose and glucose concentrations (0.1%, corresponding to 2.25 mM sucrose or 5.55 mM glucose) may also partly account for the observed results by preferentially reflecting the action of enzyme systems more active at lower substrate concentrations. Since the K_m values for invertase are from 70 to 200 mM for extracellular and from 30 to 140 mM for intracellular invertase,[24] these enzymes may have contributed little to the sucrose metabolism in these experiments. However, the K_m values for PEP-dependent phosphotransferase systems (PTS) are very low (from 40-70 μM for sucrose PTS and from 3-8 mM for glucose PTS[25,26]), and the K_m of sucrose phosphate hydrolase for sucrose phosphate, the end product of the PTS system, is 0.3 mM.[27] Thus, the substrate concentrations used here were well within the range metabolizable by *S. mutans*.

Iwami and Yamada[28] have suggested that the superior acidogenic activity of *S. mutans* relative to *S. sanguis* at low pH levels may be attributable to a more acid-tolerant ATP-dependent glucose phosphotransferase activity in *S. mutans*. The differences in acid production from sucrose and glucose at decreasing pH levels observed here may reflect variations in the acid sensitivity of the various groups' PEP or ATP-

TABLE 3
EFFECT OF DECREASING pH ON LACTIC ACID PRODUCTION FROM 0.1% SUCROSE OR GLUCOSE BY *STREPTOCOCCUS MUTANS* GENOGROUP IV STRAIN E49

		ηM Lactic Acid per 0.2 μg Protein				
pH	Sugar	2 min	5 min	10 min	20 min	30 min
6.5	sucrose	2.84	4.00	4.92	6.96	8.20
5.0	sucrose	1.36*	2.00*	3.60*	6.52	7.48
4.5	sucrose	0.24*	0.32*	0.44*	0.48*	0.52*
4.0	sucrose	0.64*	0.56*	0.52*	1.16*	0.72*
6.5	glucose	3.00	3.44	3.90	6.28	6.52
5.0	glucose	2.78	3.18	5.72	7.60+	10.76+
4.5	glucose	0.24*	0.24*	0.20*	0.30*	0.40*
4.0	glucose	0.52*	0.40*	1.86*	0.54*	0.60*

+Significantly higher than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).
*Significantly lower than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).

TABLE 4
EFFECT OF DECREASING pH ON LACTIC ACID PRODUCTION FROM 0.1% SUCROSE OR GLUCOSE BY *STREPTOCOCCUS MUTANS* GENOGROUP II STRAIN FA1

		ηM Lactic Acid per 0.2 μg Protein				
pH	Sugar	2 min	5 min	10 min	20 min	30 min
6.5	sucrose	5.44	7.00	8.76	13.52	15.16
5.0	sucrose	2.44	1.64*	3.44*	4.08*	5.40*
4.5	sucrose	3.32	2.24*	3.52*	3.56*	3.52*
4.0	sucrose	1.76	3.28	3.52	1.76*	0.40*
6.5	glucose	6.16	9.62	11.54	16.48	17.48
5.0	glucose	2.46	3.46	7.14	10.40	14.64
4.5	glucose	4.56	5.88	3.16*	3.52*	3.24*
4.0	glucose	2.14	5.38	3.90*	3.70*	5.44*

*Significantly lower than pH 6.5 ($P < 0.05$, Mann-Whitney *U* Test).

dependent sugar transport mechanisms. Further studies along these lines would be useful in clarifying the basis for these group differences.

It is apparent from these studies that *S. mutans* cells, especially those belonging to genogroup I, appear able to maintain significant acid production at those acidic pH levels which are achievable *in vivo* in carious lesions[29] and interproximal plaque.[30] This is in contrast to cells of *Streptococcus sanguis* and *Actinomyces viscosus*, which were severely inhibited at decreasing pH levels (Harper and Loesche, manuscript in preparation). The ability to produce acid at those low pH levels where enamel demineralization would proceed most rapidly may contribute significantly to the high cariogenic potential of *S. mutans*.

It should not be surprising that strains belonging to the different genogroups exhibit different metabolic activities, and the results present here may be grouped with other observations of genogroup/serotype differences. However, our observations were made with only seven strains, so that it may be premature to state that the results observed in this investigation are typical of each genogroup. Further studies using additional strains are indicated to confirm these results.

Conclusions.

Strains of *Streptococcus mutans* belonging to the four genetic groups defined by Coykendall differed in their patterns of sucrose and glucose catabolism at decreasing pH levels. The pattern of stimulation and inhibition of lactic acid production was similar in strains belonging to the same genogroup and dissimilar in strains belonging to different genogroups.

The genogroup I (serotypes *c* and *e*) strains were the most acid-tolerant, having an optimum pH for lactic acid production of pH 5.0, and maintaining acid production at an appreciable rate at pH 4.0. The genogroups II and IV strains were much more acid-sensitive than were the genogroup I strains, whereas the genogroup III strains had an intermediate level of acid sensitivity. Sucrose catabolism was frequently more pH-sensitive than glucose catabolism. These results contribute additional metabolic data emphasizing the differences among the genetic subgroups of *S. mutans*, and may partially account for the differences in *S. mutans* subgroup distribution in humans on the basis of different levels of acid-tolerance.

Acknowledgments.

We wish to thank Charles Patterson and Graham Parkinson for their valuable technical assistance, and Nancy Starks and Sandra Harris for their secretarial assistance.

REFERENCES

1. HAMADA, S. and SLADE, H.D.: Biology, Immunology and Cariogenicity of *Streptococcus mutans*, *Microbiol Rev* 44:331-384, 1980.
2. LITTLETON, N.W.; KAKEHASHI, S.; and FITZGERALD, R.J.: Recovery of Specific "Caries-Inducing" Streptococci from Carious Lesions in the Teeth of Children, *Arch Oral Biol* 15:461-463, 1970.
3. LOESCHE, W.J. and STRAFFON, L.H.: Longitudinal Investigations of the Role of *Streptococcus mutans* in Human Fissure Decay, *Infect Immun* 26:498-507, 1979.
4. TANZER, J.M.: Essential Dependence of Smooth Surface Caries on, and Augmentation of Fissure Caries by Sucrose and *Streptococcus mutans* Infection, *Infect Immun* 25:526-531, 1979.
5. HAMADA, S. and SLADE, H.D.: Mechanisms of Adherence of *Streptococcus mutans* to Smooth Surfaces *in vitro*. In: **Bacterial Adherence**, Beachey, E.H., Ed., London: Chapman and Hall, 1980.
6. HUIS in't VELD, J.H.J. and BACKER-DIRKS, O.: Intracellular Polysaccharide Metabolism in *Streptococcus mutans*, *Caries Res* 12:243-249, 1978.
7. DUMMER, P.M.H. and GREEN, R.M.: A Comparison of the Ability of Strains of Streptococci to Form Dental Plaque-Like Deposits *in vitro* with their Cariogenicity in Gnotobiotic Rats, *Arch Oral Biol* 25:245-249, 1980.
8. PERCH, B.; KJEMS, E.; and RAVN, T.: Biochemical and Serological Properties of *Streptococcus mutans* from Various Human and Animal Sources, *Acta Pathol Microbiol Scand* 82: 357, 870, 1974.
9. COYKENDALL, A.L.: Proposal to Elevate the Subspecies of *Streptococcus mutans* to Species Status, Based on their Molecular Composition, *Int J Systematic Bact* 27:26-30, 1977.
10. SHKLAIR, I.L. and KEENE, H.J.: A Biochemical Scheme for the Separation of the Five Varieties of *Streptococcus mutans*, *Arch Oral Biol* 19:1079-1081, 1974.
11. BRIGHT, J.S.; ROSEN, S.; and CHORPENNING, F.W.: Survey of the Seven Serological Types of *Streptococcus mutans* in Six-year-old Children, *J Dent Res* 56:1421, 1977.
12. THOMSON, L.A.; LITTLE, W.A.; BOWEN, W.H.; SIERRA, L.I.; AGUIRREA, M.; and GILLESPIE, G.: Prevalence of *Streptococcus mutans* Serotypes, *Actinomyces* and Other Bacteria in the Plaque of Children, *J Dent Res* 59:1581-1589, 1980.
13. HAMADA, S.; OOSHIMA, T.; TORII, M.; IMANISHI, H.; MASUDA, N.; SOBUE, S.; and KOTANI, S.: Dental Caries Induction in Experimental Animals by Clinical Strains of *Streptococcus mutans* Isolated from Japanese Children, *Microbiol Immunol* 22:301-314, 1979.
14. MINAH, G.E. and LOESCHE, W.J.: Development of Methods to Analyze Sucrose Metabolism by Small Dental Plaque Suspensions. In: Proceedings of **Microbial Aspects of Dental Caries**, Stiles, Loesche, and O'Brien, Eds., Sp. Suppl. Microbiol. Abstr. Vol. II, 1976, pp. 491-520.
15. MINAH, G.E. and LOESCHE, W.J.: Sucrose Metabolism by Prominent Members of the Flora Isolated From Cariogenic and Non-Cariogenic Dental Plaques, *Infect Immun* 17:55-61, 1977.
16. NAGEL, J.C. and KUNZ, L.J.: Simplified Storage and Retrieval of Stock Cultures, *Appl Micro* 23:837-840, 1972.
17. SYED, S.A. and LOESCHE, W.J.: Survival of Human Dental Plaque Flora in Various Transport Media, *Appl Microbiol* 24: 638-644, 1972.
18. ARANKI, A.; SYED, S.A.; KENNEY, E.B.; and FRETER, R.: Isolation of Anaerobic Bacteria from Human Gingiva and Mouse Cecum by Means of a Simplified Glove Box Procedure, *Appl Microbiol* 17:568-576, 1969.
19. UNDENFRIEND, S.; STEIN, S.; BOHLER, P.; DAIRMAN, W.; LIEMBRUBER, W.; and WEIGELE, M.: Fluorescamine: A Reagent for Assay of Amino Acids, Peptides, Proteins and Primary Amines in the Picomole Range, *Science* 178:871-872, 1972.
20. SVANBERG, M. and LOESCHE, W.J.: Implantation of *Streptococcus mutans* on Tooth Surfaces in Man, *Arch Oral Biol* 23:551-556, 1978.
21. FREEDMAN, M.L. and COYKENDALL, A.L.: Variations in the Internal Polysaccharide Synthesis Among *Streptococcus mutans* Strains, *Infect Immun* 12:475-479, 1975.
22. HAMILTON, I.R. and ELLWOOD, D.C.: Effects of Fluoride on Carbohydrate Metabolism by Washed Cells of *S. mutans* Grown at Various pH Values in a Chemostat, *Infect Immun* 19:434-442, 1978.
23. ELLWOOD, D.C.: Chemostat Studies of Oral Bacteria. In: Proceedings of **Microbial Aspects of Dental Caries**, Stiles, Loesche, and O'Brien, Eds., Sp. Supp. Microbiol. Abstr., 1976, pp. 785-798.
24. FUKUI, K.; FUKUI, Y.; and MORIYAMA, T.: Purification and Properties of Dextransucrase and Invertase from *Streptococcus mutans*, *J Bacteriol* 118:796-804, 1974.

25. SLEE, A.M. and TANZER, J.M.: Phosphoenolpyruvate-Dependent Sucrose Phosphotransferase Activity in Five Serotypes of *Streptococcus mutans*, *Infect Immun* 24:821-828, 1979.
26. ST. MARTIN, E.J. and WHITTENBERGER, C.L.: Characterization of a Phosphoenolypyruvate-Dependent Sucrose Phosphotransferase System in *Streptococcus mutans*, *Infect Immun* 24:865-868, 1979.
27. ST. MARTIN, E.J. and WHITTENBERGER, C.L.: Regulation and Function of Sucrose 6-Phosphate Hydrolase in *Streptococcus mutans*, *Infect Immun* 26:487-491, 1979.
28. IWAMI, Y. and YAMADA, T.: Rate-Limiting Steps of the Glycolytic Pathway in the Oral Bacteria *Streptococcus mutans* and *Streptococcus sanguis* and the Influence of Acidic pH on the Glucose Metabolism, *Arch Oral Biol* 25:163-169, 1980.
29. DIRKSEN, T.R.; LITTLE, M.F.; and BIBBY, B.G.: The pH of Carious Cavities – II. The pH At Different Depths in Isolated Cavities, *Arch Oral Biol* 8:91-97, 1963.
30. IMFELD, T. and LUTZ, F.: Intraplaque Acid Formation Assessed *in vivo* in Children and Young Adults, *Pediatr Dent* 2:87-93, 1980.

ANNOUNCEMENTS INDEX