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Factors Influencing Cell Shape in the Mutans Group of Streptococci

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Electron and light microscopic and growth studies of representatives of the diverse species of mutans streptococci revealed the cells to be either bacillary or coccoid in shape. Some strains changed from bacillary to coccoid if the HCO_3^-/K^+ ratio of the media was increased and from coccoid to bacillary if the ratio was decreased. Doubling times of rods and cocci were the same despite an HCO_3^-/K^+ ratio change between 0.008 and 2.84. For strain 10449S, no tested anions or cations substituted for HCO_3^- or K^+ to produce this effect, except for $B_4O_7^{2-}$. Strain 10449S grown at a high $B_4O_7^{2-}/K^+$ ratio became ellipsoid, and this phenomenon was associated with slower doubling times. Up to three incomplete septa could be observed in one rod, but no more than one incomplete septum could be observed in either ellipsoid or spherical cells. Interseptal distances were greatest in rods, shorter in spheres, and shortest in ellipses. All of the above differences were statistically significant ($P < 0.001$).

The mutans group of streptococci is indigenous to the oral cavity and is the major etiological agent of dental caries (6, 10). These bacteria were apparently named mutans because they were first found, by Clark (2), to be rods, and thus they were thought to be mutants of other streptococci. The bacillary shape of at least some of these cells was again recognized many years later (4).

Our previous study (14) demonstrated that the shape of *Streptococcus mutans* NCTC 10449S can be changed from bacillary to coccoid, and vice versa, by adjusting the salt contents of a variety of complex and defined culture media in which it is grown. Within wide ranges, the shape of this cell is not dependent on the absolute concentrations of HCO_3^-, K^+, Na^+, or phosphate; on the ratio Na^+/K^+; or on the addition (or deletion) of iron, magnesium, or manganese salts to (or from) media. The change from rod to coccoid form is growth dependent and is blocked by protein synthesis inhibitors. Shape change is not dependent on the pH change of a fermenting culture; it occurs in pH-stat controlled incubations. Rather, the shape of this cell is clearly dictated by the ratio HCO_3^-/K^+ in the growth medium. When the ratio is low (0.008), as in the modified Jordan complex medium (JM; 9, 13), the cells are bacillary; when it is high (2.84), as in the defined FMC medium (15), the cells are coccoid (14).

The present study was concerned with whether shapes of other strains among the mutans streptococcus group could be changed by manipulating this ratio, whether ions other than HCO_3^- and K^+ could similarly affect cell shapes, whether change of shape was associated with change of growth rate, and whether differences in cellular ultrastructure occurred when the cells grew in diverse shapes.

To test whether the phenomenon of changing shape in 10449S is general among the mutans group of streptococci, several strains (Table 1) representing its diverse species (3, 11) were grown anaerobically in JM (9, 13) and FMC (15) liquid media containing 0.5% glucose. Cells were examined by Gram stain and scanning electron microscopy, as previously described (14). Cell shapes are reported in terms of average length/width ratios (±standard deviation) in Table 1. Strains AHT, IB-1600, B13, LM7, and OMZ-175 were bacillary in both media, with ratios of at least 2.0. Strains Fa-1, BHT, and 6715-13WT were coccoid in both media; i.e., they had a length/width ratio of approximately 1.0. However, strain GS-5 was like 10449S, bacillary in JM and coccoid in FMC but vice versa if the HCO_3^-/K^+ ratios of these media were reversed (data not shown, but see reference 14). When FMC was modified by greatly increasing the HCO_3^-/K^+ ratio, strains AHT, B13, and LM7 became coccoid, but IB-1600 and OMZ-175 remained rod shaped.

Modification of FMC medium, e.g., to reduce HCO_3^- to 0.6 meq/liter and maintain K^+ at 6.7 meq/liter (ratio, 0.090), resulted in growth of 10449S cells, but as a mixture of rods and spheres (Fig. 1a and Table 2). These forms had statistically significantly different length/width ratios ($P < 0.001$). This FMC formulation was then used as a basal medium to which various ions were added to determine whether they might substitute for K^+ and HCO_3^- in modifying the expression of cell shape. Thus, several cations were added as chloride salts (all to 58 meq/liter); none of them had the same effect as K^+, viz., growth of only rod-shaped cells (Table 2). Several anions were added as sodium salts (all to 18 meq/liter); only HCO_3^- and $B_4O_7^{2-}$ had effects on 10449S cell shape (Table 2). In the case of $B_4O_7^{2-}$ supplementation, the cells grew, but as ellipses with length/width ratios of far less than 1.0. The length/width ratio for ellipses was different from that for cocci ($P < 0.001$; Fig. 1b and Table 2). The same phenomenon occurred with $B_4O_7^{2-}$ supplementation of JM modified to contain low K^+ (Fig. 2a).

Growth rates of 10449S were examined in six culture media: both JM and FMC, reformulations of JM and FMC made by exchanging their salts (14), and reformulations of them made by substitution of $B_4O_7^{2-}$ for nearly all of the HCO_3^- in FMC and by addition of $B_4O_7^{2-}$ to a high level in JM containing low K^+. By these procedures, JM and FMC were formulated to have the HCO_3^-/K^+ and $B_4O_7^{2-}/K^+$ ratios detailed in Table 3.

Alteration of the HCO_3^-/K^+ ratio was effected by exchange of the salts of FMC with those of JM. FMC, as normally constituted (15), contains 6.7 meq of K^+ and 19 meq of HCO_3^- (added as $NaHCO_3$) per liter, for a HCO_3^-/K^+ ratio of 2.84. JM, as normally constituted (13, 14),

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TABLE 1. Cell shapes of some strains of mutans streptococci grown in modified JM and modified FMC media

Streptococcus species and strains	Serotype (11)	Avg length/width ratio of cell shapes[a]	
		JM	FMC
S. cricetus AHT	a	B (2.13 ± 0.22; $n = 20$)[b]	B (2.41 ± 0.27; $n = 14$)[c,d]
S. rattus Fa-1	b	C (0.94 ± 0.08; $n = 22$)[c]	C (0.98 ± 0.12; $n = 28$)[c]
S. rattus BHT	b	C (1.03 ± 0.07; $n = 14$)[c]	C (1.01 ± 0.04; $n = 24$)[c]
S. mutans IB-1600	c	B (2.38 ± 0.30; $n = 19$)[b]	B (2.80 ± 0.55; $n = 26$)[c]
S. mutans 10449S	c	B (2.71 ± 0.50; $n = 26$)[c]	C (1.01 ± 0.06; $n = 36$)[c]
S. mutans GS-5	c	B (2.55 ± 0.42; $n = 22$)[b]	C (1.02 ± 0.10; $n = 20$)[c]
S. sobrinus B13	d	B (2.01 ± 0.29; $n = 27$)[b]	B (2.10 ± 0.55; $n = 12$)[c,d]
S. sobrinus 6715-13WT	g	C (1.03 ± 0.08; $n = 29$)[c]	C (1.01 ± 0.05; $n = 18$)[c]
S. mutans LM7	E	B (2.78 ± 0.32; $n = 16$)[b]	B (3.30 ± 0.72; $n = 17$)[c,d]
S. mutans OMZ-175	f	B (2.86 ± 0.34; $n = 22$)[b]	B (2.93 ± 0.60; $n = 20$)[c]

[a] The HCO_3^-/K^+ ratio is 0.008 in standardly formulated JM and 2.84 in standardly formulated FMC. Values in parentheses are means ± standard deviations. B, Bacillary; C, coccoid.
[b] Measurements were made from projections of photographs of Gram-stained smears.
[c] Measurements were made from scanning electron micrograph data.
[d] When FMC medium was modified by greatly increasing the HCO_3^-/K^+ ratio (10- to ~100-fold), these cells became coccoid.

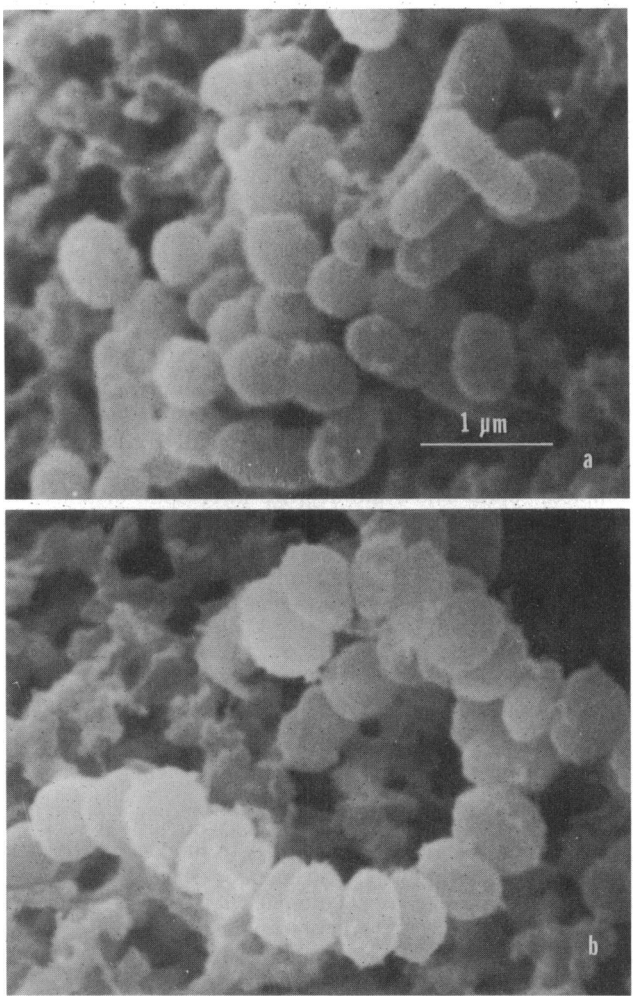

FIG. 1. Scanning electron micrographs of *S. mutans* 10449S grown in modified FMC (a) containing low HCO_3^- and low K^+, exhibiting both rod and spherical shapes, or (b) supplemented with $Na_2B_4O_7$, exhibiting ellipsoid shapes.

contains 65 meq of K^+ and 0.6 meq of HCO_3^- (added as Na_2CO_3) per liter. While filter sterilization of FMC results in no loss of HCO_3^-, autoclaving of JM does result in some loss of HCO_3^-, with an average HCO_3^-/K^+ ratio of 0.008 upon analysis (14).

In experiments with borate, $Na_2B_4O_7$ was added to a concentration of 19 meq/liter in FMC or JM. The FMC and JM were formulated at 0.6 meq of $NaHCO_3$ per liter, and the K^+ was 6.7 meq/liter in both media.

TABLE 2. Effects of ions other than K^+ and HCO_3^- on cell shape of *S. mutans* NCTC 10449S

Ions added to basal medium[a]	Cell shape(s)[b] (length/width ratio; mean ± SD)
None (control)	B (2.40 ± 0.30; $n = 9$)[c,d] C (1.05 ± 0.10; $n = 20$)[c,d]
Cations	
K^+	B (2.70 ± 0.50; $n = 36$)[c,e]
Na^+	B, C[f]
Li^+	B, C[f]
Mg^{2+}	B, C[f]
Rb^+	B, C[f]
Cs^+	B, C[f]
$Choline^+$	B, C[f]
Anions	
HCO_3^-	C (1.01 ± 0.06; $n = 36$)[c,e]
F^-	B, C[f]
Cl^-	B, C[f]
Br^-	B, C[f]
I^-	B, C[f]
NO_3^-	B, C[f]
SO_4^{2-}	B, C[f]
SCN^-	B, C[f]
$B_4O_7^{2-}$	E (0.60 ± 0.10; $n = 15$)[c,e]

[a] FMC medium modified to contain low HCO_3^- (0.6 meq/liter) and low K^+ (6.7 meq/liter). Cations were added as Cl^- salts (58 meq/liter). Anions were added as Na^+ salts (18 meq/liter).
[b] B, Bacillary; C, coccoid; E, ellipsoid.
[c] Measurements were made from scanning electron micrographs.
[d] Differences in length/width ratios between the two shapes of cells resulting from growth in the basal medium were highly significant ($P < 0.001$).
[e] Differences in length/width ratios among the three shapes of cells resulting from growth in basal medium supplemented with K^+, HCO_3^-, or $B_4O_7^{2-}$ as indicated were highly significant ($P < 0.001$).
[f] Measurements were made from projections of photographs of Gram-stained smears. Cell dimensions were not statistically different from those of 10449S grown in basal medium.

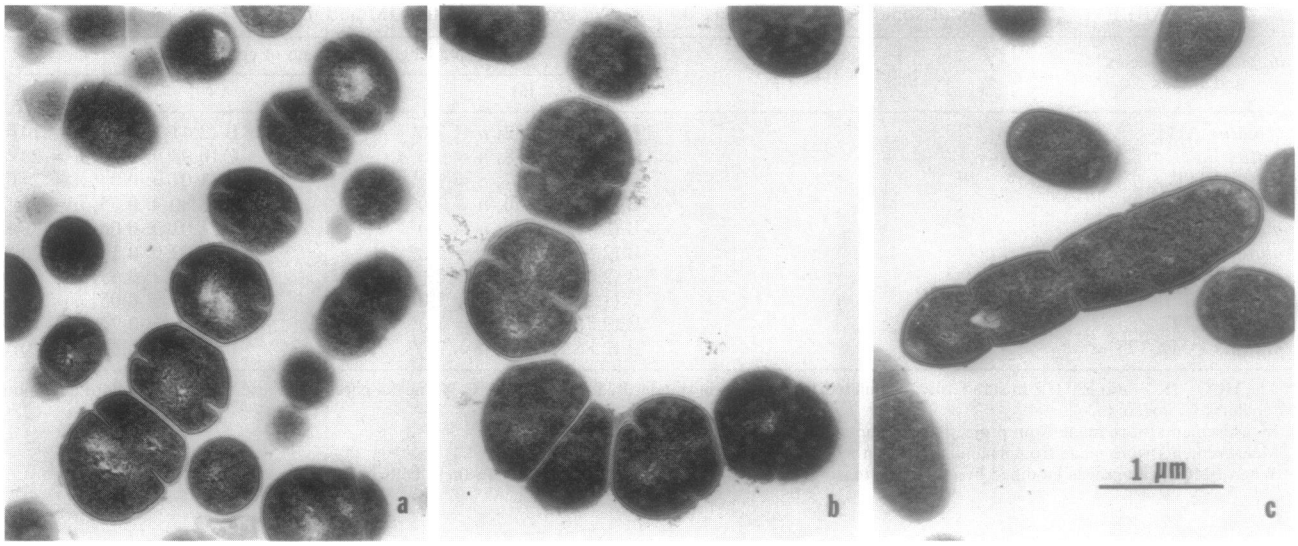

FIG. 2. Transmission electron micrographs of *S. mutans* 10449S which grew as (a) ellipses in modified JM supplemented with $Na_2B_4O_7$, (b) spheres in modified JM supplemented with $NaHCO_3$, and (c) rods in unmodified JM.

Strain 10449S was adapted to growth by repeated subculture, each subculture for a minimum of eight doubling times, in each of these six media. Growth rates were then measured turbidimetrically at 600 nm. Plots of log optical density as a function of time were made (data not shown), and correlation coefficients which described the goodness of fit of linear regression models were computed. From these data, mean doubling times were computed, with 95% confidence intervals, for each of the six growth conditions (Table 3). HCO_3^-/K^+ ratios ranging from 0.008 to 2.84 did not affect the doubling times of the cultures in either JM or FMC, despite the growth of cells as rods or spheres, respectively. Doubling times were slightly longer in FMC than in JM.

$B_4O_7^{2-}$ substitution for much of the HCO_3^- in FMC or its addition to JM containing low K^+, however, retarded cell growth rate by about twofold and was associated with ellipsoid cell shape.

To determine whether there were ultrastructural variations in the cell walls of 10449S when the cells had different shapes, thin sections of the cells were examined by transmission electron microscopy. Cells were grown anaerobically either in JM or in modified Jordan media (K_2HPO_4 reduced from 5 to 0.2 g/liter and containing either 3 g of $NaHCO_3$ or 3 g of $Na_2B_4O_7 \cdot 10H_2O$/liter). After at least two transfers in each of these media, each for at least eight generation times, cells were harvested at mid-log phase and prepared for transmission electron microscopy (7).

TABLE 3. Doubling times of *S. mutans* 10449S grown in JM and FMC media with varied salt compositions

Medium	HCO_3^-/K^+ ratio	$B_4O_7^{2-}/K^+$ ratio	Correlation coefficient (r)[a]	Mean doubling time (h) (95% confidence interval)
JM	0.008	0	0.997	1.121 (1.028–1.232)
	2.84	0	0.999	1.042 (0.983–1.108)
	0.090	2.84	0.998	2.181 (2.092–2.278)
FMC	0.008	0	0.999	1.601 (1.555–1.650)
	2.84	0	0.991	1.678 (1.543–1.839)
	0.090	2.84	0.998	2.817 (2.705–3.067)

[a] Reflects goodness of fit of plots of log optical density against time.

There was no significant difference in cell wall thickness among the different shapes (Fig. 2); however, septum formation varied. When the cells grew as rods, new septum formation occurred before complete septation of daughter cells, so that as many as three incomplete septa could be found in a single cell (Fig. 2c). However, when the cells grew as spheres or ellipses (Fig. 2b and a, respectively), the maximal number of incomplete septa was one per cell. In this case, therefore, the appearance of new septa followed completion of daughter cell formation.

Interseptal distances were measured. To avoid measurements of cells not sectioned through their poles, we studied only cells whose complete cell wall profiles could be discerned and which appeared in chains. With cocci and ellipses, distances were measured between incomplete septa and adjacent complete septa. With rods, distances were measured between incomplete septa and adjacent incomplete septa and also between incomplete septa and adjacent complete septa. Thus, the average interseptal distance for the rods was 0.60 ± 0.18 μm (n = 91), for the cocci it was 0.45 ± 0.07 μm (n = 66), and for the ellipses it was 0.27 ± 0.1 μm (n = 74). These values are different from one another (P < 0.001). As previously reported (14), the widths of rods were less than those of either cocci or ellipses.

A large number of temperature-sensitive growth or cell division mutants of *Streptococcus faecium* have been described, and the timing of the events between antibiotically induced division blocks and cell cycle events has been characterized (1). However, while surface growth models for cocci dividing in a single plane have been analyzed (5, 8), the determinants of cell shape have not. Also, an opaque colonial variant of a Lancefield group A streptococcus has been reported to be somewhat flattened or ellipsoid in shape, while the blue transparent colonial wild type of strain S23 was coccoid. However, the mechanisms of these shape variations have not been explained (12).

Data from the present study can be summarized as follows. (i) Most, but not all, strains of mutans streptococci other than 10449S evidenced coccoid or bacillary changes as a function of the HCO_3^-/K^+ ratio. (ii) 10449S may exist as cocci (at a high HCO_3^-/K^+ ratio), bacilli (at a low $HCO_3^-/$

K^+ ratio), or a combination of both distinct shapes (at low concentrations of HCO_3^- and K^+). (iii) No cations tested substituted for K^+ to induce this shape-changing effect; no anions tested substituted for HCO_3^-, except for $B_4O_7^{2-}$. (iv) 10449S grown in the presence of a high $B_4O_7^{2-}/K^+$ ratio had an ellipsoid shape such that the length/width ratio was less than 1. (v) Doubling times of rods (at a HCO_3^-/K^+ ratio of 0.008) or of cocci (at a HCO_3^-/K^+ ratio of 2.84) were the same in either FMC or JM; thus, rod and coccoid shapes were apparently not a function of growth rate. (vi) However, $B_4O_7^{2-}$ substitution for HCO_3^- in either medium slowed growth by almost twofold and was associated, although not necessarily in a causal fashion, with ellipsoid shape. (vii) When 10449S grew as rods, three incomplete septa could be found in a single cell, while only one could be found when cells were coccoid or ellipsoid. (viii) Interseptal distances were greatest for rods, shorter for cocci, and shortest for ellipses.

The mechanisms involved in the observed morphological variations of *S. mutans* NCTC 10449S and GS-5 are unknown. We hypothesize, however, that at least some mutans streptococci have a gene(s) which controls cell shape and that expression of this gene is somehow regulated by the HCO_3^-/K^+ ratio. We have undertaken studies to test this hypothesis.

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LITERATURE CITED

1. **Canepari, P., M. D. M. Lléo, G. Satta, R. Fontana, G. D. Shockman, and L. Daneo-Moore.** 1983. Division blocks in temperature-sensitive mutants of *Streptococcus faecium* (*S. faecalis* ATCC 9790). J. Bacteriol. **156**:1046–1051.
2. **Clark, J. K.** 1924. On the bacterial factor in the aetiology of dental caries. Br. J. Exp. Pathol. **5**:141–147.
3. **Coykendall, A. L.** 1983. *Streptococcus sobrinus* nom. rev. and *Streptococcus ferus* nom. rev.: habitat of these and other mutans streptococci. Int. J. Syst. Bacteriol. **33**:883–885.
4. **Coykendall, A. L., O. P. Dally, M. J. Kramer, and M. E. Beath.** 1971. DNA-DNA hybridization studies of *Streptococcus mutans*. J. Dent. Res. **50**:1131–1139.
5. **Daneo-Moore, L., and G. D. Shockman.** 1977. The bacterial cell surface in growth and division, p. 597–715. *In* G. Poste (ed.), Cell surface reviews, vol. 4. Elsevier/North-Holland Publishing Co., Amsterdam.
6. **Duchin, S., and J. van Houte.** 1978. Relationship of *Streptococcus mutans* and lactobacilli to incipient smooth surface dental caries in man. Arch. Oral Biol. **23**:779–786.
7. **Frehel, C., and A. Ryter.** 1982. Electron microscopic cytochemical study of cell-wall polysaccharides in *Bacillus subtilis* and two strains of *Bacillus megaterium*. J. Ultrastruct. Res. **81**:66–77.
8. **Higgins, M. L., and G. D. Shockman.** 1976. Study of a cycle of cell wall assembly in *Streptococcus faecalis* by three-dimensional reconstructions of thin sections of cells. J. Bacteriol. **127**:1346–1358.
9. **Jordan, H. V., R. J. Fitzgerald, and A. E. Bowler.** 1960. Inhibition of experimental caries by sodium metabisulfite and its effect on the growth and metabolism of selected bacteria. J. Dent. Res. **39**:116–123.
10. **Littleton, N. W., S. Kakahashi, and R. J. Fitzgerald.** 1970. Recovery of specific "caries-inducing" streptococci from carious lesions in the teeth of children. Arch. Oral Biol. **15**:461–463.
11. **Perch, B., E. Kjems, and T. Ravn.** 1974. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. Acta Pathol. Microbiol. Scand. Sect. B **82**:357–370.
12. **Swanson, J., and M. McCarty.** 1969. Electron microscopic studies on opaque colony variants of group A streptococci. J. Bacteriol. **100**:505–511.
13. **Tanzer, J. M., A. T. Brown, and M. F. McInerney.** 1973. Identification, preliminary characterization, and evidence for regulation of invertase in *Streptococcus mutans*. J. Bacteriol. **116**:192–202.
14. **Tao, L., J. M. Tanzer, and T. J. MacAlister.** 1987. Bicarbonate and potassium regulation of the shape of *Streptococcus mutans* NCTC 10449S. J. Bacteriol. **169**:2543–2547.
15. **Terleckyj, B., N. P. Willett, and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11**:649–655.