Role of Glycogen in Survival of *Streptococcus mitis*

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Evidence has been obtained which indicates that the possession of glycogen by *Streptococcus mitis* favors its survival during starvation.

It has been suggested that the utilization of glycogen-like polysaccharide by bacteria may favor their survival during starvation (3, 10). Evidence in support of this contention has been obtained in studies with *Aerobacter aerogenes* (6), *Escherichia coli* (7), and *Arthrobacter* species (11). The presence of a polyglucose in *Sarcina lutea*, however, adversely affected survival (2).

The synthesis of intracellular glycogen in presence of excess carbohydrate, and its rapid catabolism to lactate in the absence of exogenous carbohydrate, has been observed in *Streptococcus mitis* (5, 9). The polysaccharide appears to function as the sole reserve source of energy of this organism and may provide the cell with energy in a utilizable form (4). In the present study, therefore, the survival of glycogen-rich and glycogen-deficient cells of *S. mitis* has been compared.

Glycogen synthesis in *S. mitis* is an unstable characteristic (1, 4, 9). A glycogen-positive variant S_{25P} was derived from the glycogen-negative strain S_{23N} by methods described by Gibbons (4). Glycogen-positive strain S_3 and glycogen-negative variant S_{3N} were obtained from R. J. Gibbons, Forsyth Dental Center, Boston, Mass. Both parent strains were isolated from human dental plaque. Except for differences in glycogen synthesis, the variants were morphologically and biochemically identical to the parent strain (1, 4, 9).

The strains were cultivated aerobically at 37 C in a Trypticase-glucose broth medium (9). A 16-hr culture in maintenance medium (9) served as source of inoculum. The initial and final cell concentrations were about 2×10^7 and 3×10^9 cells/ml of growth medium, respectively. The culture sediment was washed twice at 4 C with a *p*H 6.5 buffer solution containing: K_2HPO_4 and KH_2PO_4 (0.02 M-PO_4) and NaCl (0.13 M). In some experiments, buffer was supplemented with 10^{-3} M $MgSO_4$. After taking up the sediment in the same buffer, the total cell number was deter-

mined (8), and the suspension was adjusted to the desired concentration (1×10^9 to 5×10^9 cells/ml). Periodically during aerobic storage at 37 C, samples were taken for various determinations. All procedures were performed aseptically.

Cell viability was determined by plate counting. Samples (0.1 ml) of appropriate dilutions of the suspensions in sterile saline were spread on Trypticase-soy-agar plates (BBL) with 1% glucose. The colonies were counted after 48 hr of aerobic incubation at 37 C (average of duplicate plates). Prolonged incubation, up to 7 days, did not result in higher colony counts. Gram-stained preparations of the suspensions during storage revealed no evidence of cell clumping.

The ability to ferment glucose was measured by the Warburg procedure (9), and the synthesis of glycogen was used as an indicator of overall metabolic activity. The glycogen content of the cells was estimated by measurement of the extinction of the polysaccharide-iodine complex (E_{PS-I}) at 565 nm (8). In suspensions containing 10^9 cells/ml with an E_{PS-I} value of about 0.40, glycogen accounts for approximately 50% of the total dry weight of the cell (van Houte, Ph.D. Thesis, Bronder-Offset, Rotterdam, 1967). E_{PS-I} values smaller than 0.03 for 10^9 to 5×10^9 cells/ml indicate negligible amounts of glycogen.

The survival of *S. mitis* in buffer in relation to the glycogen content of the cell is shown in Fig. 1. Logarithmic-phase cells of strain S_{25P}, rich in glycogen (5), survived well during a period in which glycogen catabolism took place. Upon depletion of the glycogen reserve, cell viability began to decrease. In contrast, glycogen-deficient cells of strain S_{25N}, cultivated under similar conditions, rapidly lost viability, and no comparable period of unchanged viability was observed. The suggestion of a contribution of glycogen to increased cell longevity was supported by the observation that storage of carbon-limited glycogen-deficient (5) cells of both strains resulted in a rapid, comparable, loss of viability (Fig. 1). Analogous results were obtained with strains S_3 and S_{3N}.

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To determine whether differences between the parent and variant strains, other than glycogen synthesis, could be responsible for the difference in survival, the following experiment was performed. Suspensions of carbon-limited stationary-phase cells of strains S_3 and S_{3N} in Mg^{2+}-supplemented buffer were prepared. One-half of the suspension of strain S_3 was preincubated aerobically with 1% glucose for 1 hr at 37 C to allow glycogen synthesis (4, 5, 8, 9); the other half was left untreated. The suspension of strain S_{3N} was

similarly preincubated with glucose. After incubation, the suspensions were centrifuged, and the sediment was washed twice in the buffer and then suspended in the same volume of buffer. Cells of strain S_3 which had synthesized glycogen during preincubation survived well in contrast to S_3 cells stored immediately after the preparation of the cell suspension (Fig. 2). The survival pattern of cells of strain S_{3N} preincubated with glucose, but unable to accumulate glycogen, was similar to that of cells of strain S_3 devoid of glycogen. In other experiments, no difference was found between the survival of cells of strain S_{3N} preincubated with glucose and cells from the same suspension immediately stored. This suggested that
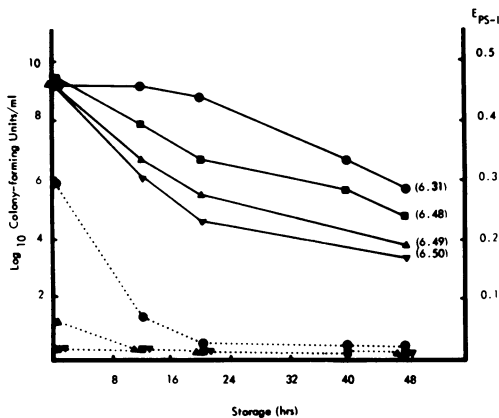

FIG. 1. *Survival of Streptococcus mitis in buffer. Strains* S_{25P} (●) *and* S_{25N} (■) *cultivated in medium with 1% glucose for 6 hr (logarithmic-phase cells). Strains* S_{25P} (▲) *and* S_{25N} (▼) *cultivated in medium with 0.1% glucose for 16 hr (stationary-phase cells). Viable count* (—); E_{PS-I} (· · · ·); *figures in parentheses, pH values of the suspensions after 48 hr of storage.*
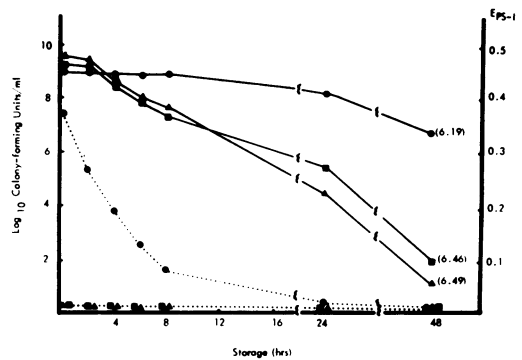

FIG. 2. *Survival of Streptococcus mitis in* Mg^{2+}-*supplemented buffer. Strain* S_3 *preincubated with glucose* (●); *strain* S_3 *not preincubated* (■); *strain* S_{3N} (▲). *Viable count* (—); E_{PS-I} (· · · ·); *figures in parentheses, pH values of the suspensions after 48 hr of storage.*

TABLE 1. *Metabolic activity of glycogen-rich and glycogen-deficient cells of Streptococcus mitis during storage in* Mg^{2+}-*supplemented buffer[a]*

		Time of storage							
		0 hr				16 hr			
Strain	Incubation with glucose	Colony-forming units/ml	EPS-I		Acid produced[b] (μmoles)	Colony-forming units/ml	EPS-I		Acid produced[b] (μmoles)
			I	II	Glucose	No substrate[c]		I	II	Glucose	No substrate[c]
S_3	+	4.7×10^9	0.30	0.40	5.61	1.36	2.9×10^9	0.03	0.15	4.00	0.30
S_3	−	4.8×10^9	0.03	0.39	8.73^d	0.20	4.0×10^5	0.02	0.02	0.40	0.21
S_{3N}	+	4.9×10^9	0.03	0.02	5.12	0.15	1.3×10^5	0.03	0.02	0.31	0.17

[a] Suspensions of cells with and without glycogen were prepared as described for the previous experiment (Fig. 2). At 0 hr, and after 16 hr of storage, samples were taken for measurement of the E_{PS-I} (I) and viable count; other samples were centrifuged, and the sediment was taken up in an equal volume of bicarbonate buffer for the Warburg experiment. At the end of the experimental period (1 hr), the E_{PS-I} was again determined by utilizing the suspensions of the flasks (II).

[b] Acid production per 2.4-ml cell suspension.

[c] Acid production from glycogen catabolism.

[d] High acid production associated with high rate of glycogen synthesis (9).

during incubation of cells of strain S_3 with glucose no changes other than glycogen deposition occurred which might be held responsible for the increased cell longevity.

The results of a typical experiment, in which the influence of storage on acid production and glycogen synthesis was studied, are shown in Table 1. The viability of glycogen-rich cells of strain S_3 decreased about 40% during a 16-hr storage period, with a concomitant decrease in the amounts of acid and glycogen produced. The viability of strains S_3 and S_{3N} without glycogen reserve after the same storage period was decreased 10,000-fold. Acid production and, in case of strain S_3, glycogen synthesis were reduced to a negligible level.

From our findings, the conclusion seems to be justified that the possession of glycogen by *S. mitis* favors its survival during starvation.

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