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Glycolytic Activity of *Streptococcus mitis* Grown In Vitro and in Gnotobiotic Animals

D. F. GORDON, JR., AND R. J. GIBBONS

Forsyth Dental Center and Harvard School of Dental Medicine, Boston, Massachusetts 02115

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The metabolic activity of organisms cultured in vitro does not necessarily reflect that occurring in vivo. Such differences have been demonstrated for *Bacillus anthracis* (H. Smith and D. W. Tempest, J. Gen. Microbiol. **17**:731, 1957), *Mycobacterium tuberculosis* (W. Segal and H. Bloch, J. Bacteriol. **72**:132, 1956), and for *Staphylococcus aureus* (P. R. Beining and E. R. Kennedy, J. Bacteriol. **85**:732, 1963). The present study compares the glycolytic activity of cells of *Streptococcus mitis* grown in gnotobiotic animals with cells cultured in vitro.

Germ-free rats (Sprague-Dawley) and mice (Charles River strain) were maintained and monocontaminated with *S. mitis* strain S3 as described by R. J. Gibbons, S. S. Socransky, and B. Kapsimalis (J. Bacteriol. **88**:1316, 1964). To obtain in vivo-grown cells, animals were sacrificed 1 to 14 weeks after inoculation and their cecal contents were suspended in 20 ml of 0.067 M potassium phosphate buffer (*p*H 7.2) by grinding in a mortar and pestle. This suspension was filtered through glass wool, centrifuged, and the cells and debris were suspended in phosphate buffer. The suspensions were adjusted to contain 50×10^6 to 180×10^6 cells per ml since preliminary experiments indicated more dense suspensions did not utilize glucose proportional to the cell number. In vitro cells of *S. mitis* were prepared from anaerobically incubated (18 hr) trypticase broth cultures containing 0.1% glucose (R. J. Gibbons, J. Bacteriol. **87**:1512, 1964). The cells were centrifuged and suspended either in cecal material from a germ-free animal or in 1% peptone buffer. Of each suspension, 5 ml was placed in duplicate serum capped tubes which were flushed with nitrogen and incubated at 37 C. To each suspension, 0.5 ml of 1% glucose was added, and 1.0-ml samples were removed at 0 and 60 min. Samples were inactivated by boiling for 5 min and then analyzed for glucose by use of glucose oxidase (Special Glucostat; Worthington Biochemical Corp., Freehold, N.J.).

TABLE 1. *Glycolytic activity of Streptococcus mitis grown in vitro and in gnotobiotic animals*

Source of cells	No. of samples	Glycolytic activity[a]		Plate counts per ml of cell suspensions ($\times 10^6$)	
		Mean	Range	Mean	Range
Gnotobiotic Animals					
Mice	6	1.60	1.43–1.81	108.5	(87.4–134)
Rats	6	2.28	1.20–3.11	84.0	(53.0–116)
Trypticase Broth					
Suspended in germ-free cecal contents of					
Mice	3	0.68	0.58–0.85	92.0	(85.0–101)
Rats	3	0.41	0.37–0.47	116.0	(82.8–179)
Suspended in buffered peptone	4	0.47	0.37–0.54	75.5	(60.0–94)

[a] Expressed in micromoles of glucose per cell per hour times 10^{-8}.

Viable plate counts were made on all cell suspensions at zero-time and after 60 min of incubation by use of Trypticase Soy Broth (BBL) with agar added as the plating medium. Total microscopic counts also were determined on cecal suspensions of three mice as described by R. J. Gibbons and B. Kapsimalis (J. Bacteriol. **93**: 510, 1967).

Glycolytic activity of in vivo-cultured cells was three to five times greater than cells cultured in vitro (Table 1) when calculated on a per cell basis. The medium used to suspend in vitro-grown cells appeared to have little effect on the metabolic

activity. Little or no growth occurred during the incubation period, since plate counts on cell suspensions incubated 0 or 60 min were ± 10% of each other. Controls indicated that free glucose was not present in germ-free cecal contents and that added glucose was not metabolized. Microscopic and viable counts for cecal material averaged 3.2×10^9 and 2.9×10^9 organisms per g, respectively, and indicated that there were few, if any, nonviable cells present. These data eliminate the possibility that nonviable metabolically active cells were responsible for the observed increased glycolytic activity. They further indicate that there need not be large numbers of dead bacteria present in the intestinal canal of animals. Similar findings have been reported for *Escherichia coli* in gnotobiotic mice (R. J. Gibbons and B. Kapsimalis, J. Bacteriol. **93**:510, 1967).

This study indicates quite clearly that the glycolytic activity of an organism, as observed in vitro, may differ significantly from that occurring under in vivo circumstances. It is interesting that the strain of *S. mitis* used in the present study has been shown to induce dental caries in gnotobiotic rats (*unpublished data*). The marked increase in glycolytic activity of this organism, as observed in vivo, may therefore be important in the etiology of this disease.

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