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Environmental Control of Glycogen and Lipid Content of *Mycobacterium tuberculosis*

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Nitrogen-limited cultures of the R_1R_v strain of *Mycobacterium tuberculosis* accumulate both glycogen and lipid as endogenous reserves. The carbon source in the medium regulates the extent of the storage material accumulation.

Studies on the composition of *Mycobacterium phlei* (1, 3) have shown the accumulation of carbohydrate and lipid storage materials to be controlled by the nutrients in the growth environment. During post-exponential growth in nitrogen-limited medium, both glycogen and lipid were stored as endogenous reserves. Endogenous reserves did not accumulate in nitrogen-excess medium. Although nitrogen limitation and similar conditions which restrict growth, but not the assimilation of carbon, have been shown to favor the synthesis of preferred reserve substances in other microorganisms (2), *M. phlei* was unique in simultaneously depositing two reserve substances.

In view of interest in the chemistry and physiological properties of the polysaccharides and lipids of tubercle bacilli, similar studies on the effect of growth environment on composition were undertaken with the R_1R_v strain of *M. tuberculosis*. This organism was grown on the surface of modified Sauton medium, harvested, and analyzed as described for *M. phlei* (1). The normal nitrogen concentration in the medium used represents the asparagine concentration (6 g/liter) most commonly used in media for the cultivation of tubercle bacilli.

In repeated experiments with all of the media tested, the per cent (dry weight) composition of the R_1R_v strain of *M. tuberculosis* was found to remain constant during exponential growth at 8.3 ± 0.1 SD for nitrogen, 13.1 ± 0.3 SD for lipid, 10.7 ± 0.9 SD for total carbohydrate, and 0.4 ± 0.2 SD for glycogen. This lipid and carbohydrate content apparently represents the minimal value essential for cell structure and metabolism. Contents of lipid and polysaccharide above these amounts were considered to be non-essential endogenous reserves.

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M. tuberculosis accumulated large reserves of both glycogen and lipid during post-exponential growth on nitrogen-limited glycerol medium (Table 1). The glycogen accumulation was reduced by increasing the initial nitrogen concentration of the medium, and glycogen remained low and constant throughout growth in nitrogen-excess glycerol medium. Similar accumulations of glycogen and lipid occurred during post-exponential growth on nitrogen-limited medium with glucose as a carbon source (Table 2). When the initial nitrogen concentration of this medium was increased, storage material was reduced, and neither glycogen nor lipid accumulated in *M. tuberculosis* grown on nitrogen-excess glucose medium. Because of inherent difficulties in duplicating inocula for surface cultures (3), the maximal levels of accumulated lipid and carbohydrate were not consistent in repeated experiments. In all replications of these experiments, however, the patterns of accumulation and the effects of nitrogen concentration and carbon source were identical to those described above.

The results of these analyses have shown that nutrition can control the polysaccharide and lipid content of *M. tuberculosis*. The accumulation of glycogen and lipid during growth in nitrogen-limited medium and not in nitrogen-excess medium suggests that the synthesis of these endogenous reserves is controlled primarily by the ratio of nitrogen to carbon in the environment. Since accumulations of storage materials are consistently lower in glucose-grown than in glycerol-grown cells, it appears that the carbon source also regulates the extent of accumulations in *M. tuberculosis*. The deposition of two reserve materials appears characteristic of the mycobacteria. With one exception, these results duplicate the observations on accumulation of storage material in *M. phlei*. The persistent accumulation of lipid in *M. tuberculosis* grown

TABLE 1. *Yield of organism and storage material content of Mycobacterium tuberculosis grown on asparagine-glycerol media at three concentrations of the nitrogen substrate*[a]

Nitrogen concn of growth medium	Age of culture (days)	Yield of cells (mg/100 ml of culture, dry wt)	Constituents (% dry wt)			
			Nitrogen	Lipid	Total carbohydrate	Glycogen
Nitrogen-limited (60 mg of N/100 ml of medium)	0	1	8.0	13.2	9.7	0.2
	12	215	7.8	15.7	10.0	0.4
	19	590	6.9	18.5	16.7	5.0
	22	730	6.5	22.1	25.2	14.1
Normal (120 mg of N/100 ml of medium)	0	1	8.0	13.2	9.7	0.2
	12	372	8.1	13.2	10.7	0.3
	19	716	7.8	15.3	12.1	3.7
	22	921	7.8	19.7	17.8	7.9
	26	1,311	7.3	22.6	18.9	8.2
Nitrogen-excess (240 mg of N/100 ml of medium)	0	1	8.0	13.2	9.7	0.2
	12	230	8.3	12.9	9.6	0.2
	19	490	7.3	15.6	5.1	<0.1
	22	520	7.8	18.4	5.4	<0.1

[a] Results of a typical experiment. Inocula for all media came from the same surface culture. Cells pooled from at least three replicate cultures were used for each determination.

TABLE 2. *Yield of organism and storage material content of Mycobacterium tuberculosis grown on asparagine-glucose media at three concentrations of the nitrogen substrate*[a]

Nitrogen concn of growth media	Age of culture (days)	Yield of cells (mg/100 ml culture, dry wt)	Constituents (% dry wt)			
			Nitrogen	Lipid	Total carbohydrate	Glycogen
Nitrogen-limited (60 mg of N/100 ml of medium)	0	1	8.4	13.1	11.5	0.5
	13	116	8.3	13.4	11.8	0.6
	20	405	7.7	11.7	14.0	2.8
	23	610	5.7	12.1	22.1	7.9
	27	736	5.9	13.4	20.3	5.1
Normal (120 mg of N/100 ml of medium)	0	1	8.4	13.1	11.5	0.5
	13	226	8.6	12.9	11.4	0.5
	20	619	8.7	12.7	11.9	0.7
	23	786	8.6	13.6	16.0	3.2
	27	928	8.2	15.2	13.7	1.1
Nitrogen-excess (240 mg of N/100 ml of medium)	0	1	8.4	13.1	11.5	0.5
	13	151	8.7	12.7	10.7	0.3
	20	262	8.9	11.8	8.3	0.2
	23	350	8.8	12.6	7.7	0.1
	27	390	8.6	13.4	7.3	<0.1

[a] Results of a typical experiment. Inocula for all media came from the same surface culture. Cells pooled from at least three replicate cultures were used for each determination.

on glycerol cannot be explained by the present data but will be studied in a more detailed analysis of the regulation of storage material accumulation in the mycobacteria.

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