JOURNAL OF BACTERIOLOGY, Dec., 1966
Vol. 92, No. 6
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Initial Steps in the Metabolism of Glycerol by *Mycobacterium tuberculosis*

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Received for publication 29 July 1966

Although glycerol is the main carbon source usually employed in the culture of mycobacteria, there is some apparent conflict in the literature about whether its metabolism in these organisms involves phosphorylation followed by dehydrogenation or vice versa (G. J. E. Hunter, Biochem. J. **55**:320, 1953; D. S. Goldman, J. Bacteriol. **86**:30, 1963). This note reports preliminary work to resolve this question.

Mycobacterium tuberculosis BCG was grown in a modified Sauton medium (J. Youatt, Australian J. Exptl. Biol. Med. Sci. **36**:223, 1958) in conical flasks at 37 C. Cells were incubated under stationary conditions for 4 days and then on a rotary shaker for 4 days (young cultures), or they were incubated under stationary conditions for 4 weeks (old cultures). Extracts were prepared by suspending 0.4 g (dry weight) of cells in 8 ml of 0.02 M phosphate buffer (*p*H 7.0). The cells were treated with a 60-w ultrasonic disintegrator for 5 min below 10 C, and were then centrifuged at 20,000 × *g* for 30 min; the sediment was discarded. When dialysis was done, it was against deionized water at 5 C for 24 hr. Protein was estimated by the biuret method.

The tetrazolium method of measurement of dehydrogenase activity was based on that of Lin et al. (Proc. Natl. Acad. Sci. U.S. **48**:2145, 1962). The assay mixture contained 0.3 ml of extract, 1 mmole of phosphate buffer (*p*H 7.0), 100 μmoles of glycerol or glycerol-3-phosphate, 2 mg of KCN, 0.1 mg of phenazine methosulfate, and 0.1 mg of 3-(4,5-dimethylthiazolyl 1-2)-2,5-diphenyltetrazolium bromide (MTT) in a total volume of 3 ml. A 0.5-μmole amount of nicotinamide adenine dinucleotide (NAD) or NAD phosphate (NADP) was added where specified. The reaction (at 20 C) was followed at 550 mμ. Blank (no substrate) rates were subtracted. A millimolar extinction coefficient of 11 for the formazan was used.

In the assay for NAD- or NADP-dependent dehydrogenases, about 0.3 ml of extract was incubated at 37 C with 400 μmoles of hydrazine buffer (*p*H 9.1), 200 μmoles of glycerol or glycerol-3-phosphate, and 0.5 μmole of NAD or NADP in a total volume of 3 ml. The reaction was followed at 340 mμ, and blank (no substrate) rates were subtracted.

The results in Table 1 show that extracts from BCG cultures, whether young or old, contained glycerol-3-phosphate dehydrogenase activity, indicating that in this organism the metabolism of glycerol is at least partly by phosphorylation before dehydrogenation. This agrees with findings with saprophytic mycobacteria (Hunter, Biochem. J. **55**:320, 1953), and renders rather surprising the reported absence of glycerol-3-phosphate dehydrogenase from *M. tuberculosis* H37Ra (D. S. Goldman, J. Bacteriol. **86**:30, 1963).

The fact that higher activity was found by tetrazolium reduction than by NAD or NADP reduction (in spite of the higher temperature employed when measuring the latter two) and the slightness of the effect of dialysis on the former activity both suggest that most of the activity was due to an NAD- and NADP-independent dehydrogenase (E.C.1.1.99.5), but the results indicate that some NADP-dependent glycerol-3-phosphate dehydrogenase (E.C.1.1.1.8) may also have been present. It is frequently the case that forward and reverse reactions in metabolic pathways are catalyzed by different enzymes. Hence, an NADP-dependent dehydrogenase may exist to catalyze the reverse reaction from triose phosphate, since the standard free energy change of the reaction of dihydroxyacetone phosphate with reduced NADP ($NADPH_2$) is about −6 kcal at *p*H 7, whereas an NADP-independent dehydrogenase may bring about the forward reaction by employing a hydrogen acceptor of higher standard redox potential than NADP.

In view of the discovery of glycerol dehydrogenase activity dependent on NADP in *M. tuberculosis* H37Ra (Goldman, J. Bacteriol. **86**:30, 1963) and on NAD in *M. smegmatis* (F. G. Winder and C. O'Hara, Biochem. J. **90**:122, 1964), a search for this activity in BCG was carried out. A variety of methods of extraction and assay were tried, and the methods described above were the most successful. The results in Table 1

TABLE 1. *Glycerol-3-phosphate and glycerol dehydrogenase activities in cell-free extracts of young and old cultures of Mycobacterium tuberculosis BCG*

Assay method	Type of culture	Treatment of extract	Hydrogen acceptor	Glycerol-3-phosphate dehydrogenase[a]	Glycerol dehydrogenase[a]
Tetrazolium reduction	Young	None	MTT	2.32	0.01
		Dialyzed	MTT	1.98	—
		Dialyzed and NADP added	MTT	2.36	—
	Old	None	MTT	1.66	0.01
NAD or NADP reduction	Young	None	NAD	0.17	0.00
		None	NADP	0.98	0.04
	Old	None	NAD	0.02	1.51
		None	NADP	0.20	0.17

[a] Expressed as millimicromoles per minute per milligram of protein.

show that no more than traces of glycerol dehydrogenase activity of any sort were present in extracts of young shaken cultures, but that decided NAD-dependent activity existed in the extracts of old surface cultures. It is possible that the appearance of this activity in the latter cultures was due to low oxygen tension through much of their volume; *Streptococcus faecalis* and *Aerobacter aerogenes* contain an NAD- and NADP-independent glycerol-3-phosphate dehydrogenase during aerobic growth on glycerol, but have in its place an NAD-dependent glycerol dehydrogenase when growth is anaerobic (N. J. Jacobs and P. J. VanDemark, J. Bacteriol. **79:** 532, 1960; E. C. C. Lin, A. P. Levin, and B. Magasanik, J. Biol. Chem. **235:**1824, 1960).

This investigation was supported by Public Health Service grant AI-02842 from the National Institute of Allergy and Infectious Diseases, and by Arthur Guinness Son & Co. (Dublin) Ltd.