or suspensions of individual or mixed fractions in a phosphate buffer (pH 6.8) containing 0.9% sodium chloride, at room temperature for 1 to 2 weeks. Subcultures were made on blood agar, and then again in solutions of mycobacterial fractions.

Starting with the third series of subcultures, the cultures were centrifuged at 17,000 × *g* for 10 min. Collected supernatants were recentrifuged at 27,000 × *g* for 10 min, then concentrated to one-half to one-third volume at room temperature by the use of a fan. Filtrates were serially diluted and tested against an equal volume of individual fractions of mycobacteria diluted 1:20,000 or 1:50,000 in a complement-fixation test, as described by Kwapinski and Snyder (*The Immunology of Rheumatism*, Appleton-Century-Crofts, New York, 1961). Adequate controls of fraction solutions and filtrates at double concentrations were set up simultaneously. Some fractions were also tested against the culture filtrates in the Ouchterlony test.

Results based on 15 series of tests showed that filtrates of *Candida* and *Rhodotorula* grown in the presence of nucleoprotein fractions, and particularly in the solutions of combined nucleoprotein and carbohydrate or nucleoprotein and phospholipid fractions, gave regularly a strong complement fixation when tested against nucleoprotein and polysaccharide, but not against phospholipid, fractions. In the diffusion precipitation test, only nucleoprotein fractions reacted, and less regularly, with the culture filtrates.

This preliminary work is being followed by larger series of similar tests and by investigations to elucidate the nature and mechanism of these phenomena, which have characteristics similar to those of a true serological reaction.

α-HYDROXYGLUTARATE: PRODUCT OF AN ENZYMATIC BETA-CONDENSATION BETWEEN GLYOXYLATE AND PROPIONYL-COENZYME A

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Reeves and Ajl (J. Bacteriol. **84**:186, 1962) reported the formation of α-hydroxyglutarate from glyoxylate and propionyl-coenzyme A (CoA). This condensation is catalyzed by an enzyme obtained from *Escherichia coli* which had been grown aerobically in a mineral-salts medium containing propionate as the sole carbon source. Subsequent studies in our laboratory have been concerned with the role of glyoxylate in the growth of bacteria adaptively grown in media containing butyrate or valerate as the sole carbon source. It has been shown that in extracts obtained from butyrate-grown cells there is an enzyme which catalyzes the condensation between glyoxylate and butyryl-CoA to form β-ethylmalate (Rabin, Reeves, and Ajl, Bacteriol. Proc., p. 104, 1963). Further, it has been reported that valerate-adapted cells contain an enzyme which catalyzes the condensation between glyoxylate and valeryl-CoA to form *n*-propylmalate (Imai, Reeves, and Ajl, Bacteriol. Proc., p. 103, 1963).

Whereas β-ethylmalate and *n*-propylmalate are the products of a condensation between glyoxylate and the alpha-carbon of butyryl-CoA and valeryl-CoA, respectively, α-hydroxyglutarate is the product of a condensation between glyoxylate and the beta-carbon of propionyl-CoA. The expected product of an alpha-carbon condensation, β-methylmalate, has never been demonstrated in reaction mixtures containing glyoxylate and propionyl-CoA. It occurred to us that either (i) α-hydroxyglutarate was indeed the direct condensation product even though an alpha-carbon condensation might appear more reasonable or (ii) β-methylmalate is formed initially and then undergoes an isomerization to yield α-hydroxyglutarate. If the latter were true, then it should be possible to demonstrate the formation of α-hydroxyglutarate from β-methylmalate by extracts obtained from propionate-grown cells.

Reaction mixtures (3.0 ml), each containing 100 μmoles of tris(hydroxymethyl)aminometh-

ane·HCl buffer (pH 8.0), 10.0 μmoles of $MgCl_2$, and 0.5 ml of crude extract (3.1 mg of protein), were incubated at 37 C for 4 hr under anaerobic conditions with the following additions: (A) 13.0 μmoles of sodium glyoxylate and 12.0 μmoles of propionyl-CoA; (B) 20.0 μmoles of disodium β-methylmalate, 10 μmoles of adenosine triphosphate, and 5 μmoles of CoA-SH; (C) 10 μmoles of β-methylmalyl-CoA.

Propionyl-CoA was prepared according to the method of Simon and Shemin (J. Am. Chem. Soc. **75**:2520, 1953) and β-methylmalyl-CoA by the method used by Trans and Brody (J. Am. Chem. Soc. **82**:2972, 1960) for the synthesis of methylmalonyl-CoA. β-Methylmalate was prepared according to the method of Scherp (J. Am. Chem. Soc. **68**:912, 1946). The acyl-CoA derivatives were assayed by the hydroxamate method of Lipmann and Tuttle (J. Biol. Chem. **159**:21, 1945). Protein was determined by the method of Warburg and Christian (Biochem. Z. **310**:384, 1945).

The reactions were terminated by the addition of 0.2 ml each of 50% H_2SO_4 and 15% sodium tungstate. Protein was removed by centrifugation, and the supernatant fluid was extracted continuously with ether for 36 hr. The ether was then evaporated off, the residue was dissolved in a small amount of water, and samples were spotted on Whatman no. 1 filter paper. Chromatography was ascending at 23 C. Spots were located (after autoclaving to remove swamp acids) by spraying with 0.04% bromcresol green (pH 11) in 95% ethanol. The results are shown in Table 1.

As can be seen in Table 1, the solvent systems employed afford good resolution of α-hydroxyglutarate and β-methylmalate. From papers developed in these solvent systems, the following observations were made: reaction mixture A, containing glyoxylate and propionyl-CoA as substrates, showed a spot corresponding to α-hydroxyglutarate and no spot at the R_F of β-methylmalate; reaction mixtures B and C, containing β-methylmalate and β-methylmalyl-CoA, respectively, as substrates exhibited a spot corresponding to authentic β-methylmalate but no spot at the R_F of α-hydroxyglutarate. These results strongly suggest that α-hydroxyglutarate is enzymatically produced by a condensation of glyoxylate with the beta-carbon of propionyl-CoA and that β-methylmalate is not an intermediate in this reaction. Isotope dilution experiments are currently being conducted to confirm these results and will be published elsewhere.

TABLE 1. *Chromatographic resolution of α-hydroxyglutarate and β-methylmalate**

Substrate	Solvent system†					
	1	2	3	4	5	6
α-Hydroxyglutarate..	0.49	0.47	0.67	0.67	0.69	0.34
β-Methylmalate.....	0.57	0.54	0.73	0.71	0.75	0.44

* Results are expressed as R_F values.

† Solvent 1: ethyl acetate–formic acid–water (10:1:4). Solvent 2: ethyl acetate–acetic acid–water (4:1:5). Solvent 3: ether–formic acid–water (5:2:1). Solvent 4: *n*-butanol–formic acid–water (4:1:5). Solvent 5: ethyl acetate–formic acid–water (10:2:3). Solvent 6: ether–benzene–formic acid–water (21:9:7:2).

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SPORE FORMATION AND HEAT RESISTANCE IN *RHIZOBIUM*

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Bissett (J. Gen. Microbiol. **7**:232, 1952; **20**:89, 1959) reported sporogenic strains of *Rhizobium* able to withstand heating at 80 C for 20 to 30 min, and suggested a possible relationship between these bacteria and the genus *Bacillus*. The following results obtained in this laboratory.

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