prior alkaline hydrolysis of the incubation mixture and were concluded to be in the form of free (unesterified) acids.

The enzyme shows optimum pH 6.5–6.7 and is completely specific for NADPH. Determined at 20°C, K_m values of 6.0×10^{-5} M and 1.9×10^{-5} M were obtained for malonyl-CoA and NADPH respectively. No enzyme preparation has been obtained free from malonyl-CoA decarboxylase activity, but an apparent K_m of 7.0×10^{-5} M has been derived for acetyl-CoA. The variation of kinetic parameters with temperature is under investigation.

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Effects of Isoniazid on Mycolic Acid Synthesis in *Mycobacterium tuberculosis* and on its Cell Envelope

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Indirect evidence has suggested that isoniazid has an early effect on the cell envelope of B.C.G.* (F. G. Winder & S. A. Rooney, unpublished work). The findings outlined below provide more direct evidence for this.

When growing B.C.G. was exposed to $10 \mu\text{g}$ of isoniazid/ml for 6h, harvested and treated with a 60 W ultrasonic generator for 20min or with a 100 W generator for 10min, and the cell walls were prepared as described by Kotani, Kitaura, Hirano & Tanaka (1959), the yield of walls from isoniazid-treated cells was about twice that from controls. When shorter periods of disintegration were used the difference in yield of walls was even more marked. These results indicate that isoniazid treatment leads to alteration of the cell envelope of B.C.G., resulting either in its easier rupture or in a reduced tendency to clump.

When growing B.C.G. was exposed simultaneously to $1 \mu\text{g}$ of isoniazid/ml and [^{14}C]glycerol (as major carbon source) for 12h, the isoniazid had no appreciable effect on the total incorporation of radioactivity into the cells. However, it decreased the specific radioactivity of the cell walls, prepared by the method

of Kotani *et al.* (1959), by about one-half, indicating a specific effect on the formation of these.

When growing B.C.G. was exposed to $1 \mu\text{g}$ of isoniazid/ml and [^{14}C]glycerol for 6h and harvested, and unbound lipids were removed by extraction with ethanol-diethyl ether (3:1, v/v) at 50°C, the cells saponified in methanol-benzene (1:1, v/v) containing KOH (2.5%, w/v) for 24h with refluxing, the fatty acids extracted with diethyl ether, mycolic acids precipitated from the extract with 2vol. of ethanol and then redissolved and reprecipitated, the specific radioactivity of the mycolic acids was one-seventh of that of the mycolic acids from control cells. This result indicates that isoniazid has an early, and perhaps primary, effect on mycolic acid synthesis.

It is suggested that the effect on mycolic acid synthesis leads to interference with the synthesis of the mycolic acid-containing lipopolysaccharides that are major components of the cell envelope of mycobacteria (Imaeda, Kanetsuna & Galindo, 1968). This hypothesis enables us to account for the effects of isoniazid on the alkali-extractable polysaccharides of B.C.G. (Winder & Rooney, 1968a) and for certain effects on its lipids (Winder & Rooney, 1968b).

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Threonine Metabolism in a Strain of *Bacillus subtilis*

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Published evidence suggests that threonine may be catabolized by micro-organisms via a number of alternative routes. The initial step may be catalysed by a dehydratase (Umberger & Brown, 1957), an aldolase (Dainty & Peel, 1966; Dainty, 1967) or a dehydrogenase (Elliott, 1959). In the last case, the product 2-amino-3-oxobutyrates may be either enzymically split to glycine and acetyl-CoA (McGilvray & Morris, 1969) or spontaneously decarboxylated to give aminoacetone. Higgins, Pickard & Turner (1968) have shown that aminoacetone is rapidly metabolized by specially selected bacteria, and we now present evidence that threonine catabolism via aminoacetone occurs in a strain of *Bacillus subtilis* grown on a complex natural medium.

*Abbreviation: B.C.G., Bacille Calmette Guérin.

Aminoacetone was formed from L-threonine in cell-free extracts of *B. subtilis* grown on nutrient broth. The amino ketone was then alternatively reduced to 1-aminopropan-2-ol or transaminated to methylglyoxal. Whereas 1-aminopropan-2-ol was apparently not further metabolized, methylglyoxal was oxidized to pyruvate, either directly or indirectly via D-lactate or lactaldehyde.

Enzymes of the 'amino ketone pathway' of threonine metabolism that were studied include L-threonine dehydrogenase, aminoacetone aminotransferase (Higgins, Turner & Willetts, 1967), aminoacetone reductase, 1-aminopropan-2-ol dehydrogenase (Turner, 1966), methylglyoxal oxidase (Monder, 1965; Higgins & Turner, 1969), methylglyoxalase (Racker, 1952; Higgins & Turner, 1969), methylglyoxal reductase (Ting, Miller & Sellinger, 1965), D-lactate dehydrogenase (Brin & Dunlop, 1965) and lactaldehyde oxidase. Additional enzymes studied were L-threonine dehydratase, L-threonine aldolase, 2-amino-3-oxobutyrates-CoA ligase (McGilvray & Morris, 1969), the 'glycine-cleavage system' (Sagers & Gunsalus, 1961), L-serine hydroxymethyltransferase and L-serine dehydratase. The properties of these enzymes partially purified from *B. subtilis* are consistent with the operation of the amino ketone pathway. Radiotracer experiments with L-[U-¹⁴C]threonine support this conclusion, as also do growth and enzyme-induction results.

The effect of growth conditions on enzyme activities was examined with simple synthetic media. The aminoacetone-dependent pathway predominated in *B. subtilis* grown on such media containing relatively low concentrations of L-threonine. Whereas L-threonine dehydrogenase, methylglyoxalase and methylglyoxal oxidase were constitutive, at high activities, the formation of all other enzymes was markedly induced by the amino acid. The additional development of a threonine aldolase-dependent pathway was induced by higher threonine concentrations. Threonine aldolase and the enzymes of glycine metabolism via serine were all markedly affected. Catabolic pathways involving threonine dehydratase or 2-amino-3-oxobutyrates-CoA ligase did not appear to operate in *B. subtilis*, even when L-threonine served as the major source of carbon for growth.

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2,3-Dihydroxybenzoate 3,4-Oxygenase from *Pseudomonas fluorescens*: Determination of the Site of Ring Cleavage with a Substrate Analogue

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The oxidation of 2,3-dihydroxybenzoate has been shown to proceed in fungi by decarboxylation to catechol and an intradiol oxygenative cleavage of this product to yield eventually β -oxoadipate (Terui, Enatsu & Tabata, 1961; Subba Rao, Moore & Towers, 1967). It has been shown that *Pseudomonas fluorescens* 23D-1 is able to cleave the benzenoid ring of 2,3-dihydroxybenzoate by an extradiol cleavage to yield α -hydroxymuconic semialdehyde and carbon dioxide (Ribbons, 1966a). Catechol was excluded as an intermediate in this transformation, even though α -hydroxymuconic semialdehyde is also the product of the extradiol ring cleavage of catechol by catechol 2,3-oxygenase (Dagley, Evans & Ribbons, 1960; Kojima, Itada & Hayaishi, 1961). There was, however, doubt about the site of the extradiol cleavage of 2,3-dihydroxybenzoate, since α -hydroxymuconic semialdehyde could arise by cleavage of C₍₁₎-C₍₂₎ or C₍₃₎-C₍₄₎ bonds (Ribbons & Watkinson, 1968).

An analogue of 2,3-dihydroxybenzoate, 2,3-dihydroxy-*p*-toluate, has been isolated as co-oxidation product from *p*-xylene fermentations (Raymond, Jamison & Hudson, 1967) and a sample of this compound (kindly provided by R. Raymond) was tested as a substrate for 2,3-dihydroxybenzoate oxygenase. 2,3-Dihydroxy-*p*-toluate is oxidized by 2,3-dihydroxybenzoate-induced cells and extracts of *Ps. fluorescens* with the consumption of 1 mol of oxygen and release of 1 mol of carbon dioxide and the stoichiometric formation of 2,6-dioxoheptenoic acid; this acid is not further metabolized by *Ps. fluorescens*. The isolated product was characterized by

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