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evidence has suggested that isoniazid has an early effect on the cell envelope of B.C.G.* (F. G. Winder & S. A. Rooney, unpublished work).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-oxobutylate 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.