grown on mandelate into benzoate+mandelate-salts medium gave rapid growth in two distinct phases. In this case the utilization of mandelate was immediate but arithmetic, and accelerated only after exhaustion of the benzoate. When benzoate was added to a culture growing in mandelate-salts medium, the utilization of mandelate became arithmetic but was not inhibited. Utilization of mandelate during growth was also suppressed by succinate or catechol. No substantial feedback inhibition was observed of mandelate oxidation in washed-cell suspensions or of enzyme activities in cell-free preparations. Benzoate repressed the synthesis of L-mandelate dehydrogenase in a mutant strain devoid of benzoate oxidase activity. It seems likely therefore that the mandelate pathway of bacterium N.C.I.B. 8250 is subject to multi-sensitive end-product repression of the type observed in *Pseudomonas putida* (Mandelstam & Jacoby, 1965; Stevenson & Mandelstam, 1965).

Growth in benzyl alcohol+benzoate-salts medium gave a different pattern of results. If the inoculum was grown on benzoate then diauxie was observed, with benzoate utilized before the benzyl alcohol. If, however, the inoculum was grown on benzyl alcohol there was no diauxie and utilization of both substrates was exponential. By analogy with a similar situation in *Pseudomonas aeruginosa* growing on glucose+citrate (Hamilton & Dawes, 1961; Hamlin *et al.*, 1967), the different behaviour in benzoate+benzyl alcohol-salts medium depending on the nature of the inoculum suggests the possibility of a specific and inducible transport mechanism for benzyl alcohol in this organism.

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Possible Transport Mechanisms for Mandelate and Benzoate in Bacterium N.C.I.B. 8250

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Relatively little work has been done on the mechanism of entry of non-amino aromatic compounds into bacterial cells. Hegeman (1966) found no evidence for a permease for mandelate in *Pseudomonas putida*. We have developed an assay for measuring pools of certain aromatic compounds in cells of bacterium N.C.I.B. 8250, and have obtained evidence to indicate that benzoate and mandelate enter this organism by facilitated diffusion.

Cell suspensions containing carboxy-¹⁴C-labelled substrates were incubated at 30°C with aeration. Samples were taken at intervals, washed on filters under rigorously defined conditions and their radioactivities counted by liquid-scintillation spectrometry. Corrections were made for interstitial and bound material, and the concentrations of intracellular material were calculated.

Various degrees of permeability barrier to mandelate were observed in cells of bacterium N.C.I.B. 8250 that had been grown under conditions that did not induce the enzymes for mandelate oxidation. For instance, mandelate entered cells grown in nutrient broth at a low but detectable rate and entered cells grown on benzyl alcohol even more slowly. No pool of mandelate could be detected in cells of bacterium N.C.I.B. 8250 induced to, and incubated in the presence of, mandelate. With a mutant strain lacking L-mandelate dehydrogenase and grown in the presence of phenylglyoxylate (Livingstone & Fewson, 1971), however, the intracellular concentration of mandelate rapidly reached the extracellular concentration.

No permeability barrier to benzoate could be detected in non-induced cells, but evidence for facilitated diffusion was obtained from exit counterflow experiments (see, e.g., Wong & Wilson, 1970). Salicylate was not decarboxylated by cells that had been grown on benzoate, but did rapidly enter these cells to attain and maintain the extracellular concentration. The addition of benzoate to cells in suspension and containing salicylate caused an exit of salicylate against a concentration gradient. Further, the decarboxylation of 4-fluorobenzoate was prevented by benzoate. Presumably this interaction occurred at the cell membrane, since no 4-fluorobenzoate could be detected inside the cells.

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Mechanism of Inhibition of Fatty Acid Oxidation by Pent-4-enoic Acid: Evidence Against the Coenzyme A-Depletion Hypothesis

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Pent-4-enoic acid strongly inhibits oxidation of fatty acids, pyruvate and 2-oxoglutarate (Senior & Sherratt, 1968; Senior *et al.*, 1968), and these inhibitions depend on the formation of intramitochondrial pent-4-enoyl-CoA (Holland & Sherratt, 1969; Sherratt *et al.*, 1971). Bressler *et al.* (1969) proposed that sequestration of CoA as pent-4-enoyl-CoA and its metabolites impairs CoA-dependent oxidations. This conclusion was apparently supported by the quantitative work of Fukami & Williamson (1971), who found that incubation of rat liver mitochondria with pent-4-enoic acid caused rapid lowering of the free CoA concentration and elevation of acid-soluble acyl-CoA concentrations. However, this explanation overlooked the fact that some other simple fatty acids that readily form CoA derivatives and that inhibit pyruvate and 2-oxoglutarate oxidation as much as pent-4-enoic acid do not inhibit fatty acid oxidation as strongly (Senior & Sherratt, 1968; Senior *et al.*, 1968).

The effects of pent-4-enoic acid and of some simple fatty acids on the concentrations of the free and acylated forms of CoA in rat liver mitochondria were determined (Tubbs & Garland, 1964; Allred & Guy, 1969). It was confirmed that 0.1 mM-pent-4-enoic acid causes rapid depletion of free CoA together with an increase of acid-soluble acyl-CoA and some increase in acetyl-CoA concentrations (due to a self-limiting oxidation of pent-4-enoyl-CoA; Sherratt *et al.*, 1971). However, 0.1 mM-cyclopropanecarboxylic acid, which does not inhibit β -oxidation strongly, caused a similar fall in the CoA concentration and an increase in acid-soluble acyl-CoA concentrations. 0.1 mM-*n*-Pentanoic acid also caused similar changes, although 0.1 mM-octanoic acid (which is rapidly oxidized) only lowered the free CoA concentration by about 50%.

Very little free CoA is found in mitochondria during rapid oxidation of palmitoyl-carnitine (Garland *et al.* 1965, Fukami & Williamson, 1971). This, and the fact that non-inhibitory concentrations of cyclopropanecarboxylic acid or *n*-pentanoic acid cause similar lowering of free CoA concentrations,

confirm that β -oxidation has a very high affinity for CoA. These results indicate that lowering of free CoA concentrations by some simple fatty acids may impair oxidation of pyruvate or 2-oxoglutarate, but that this depletion is not the primary cause of inhibition of β -oxidation by pent-4-enoic acid.

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Mechanism of Inhibition of Fatty Acid Oxidation by Pent-4-enoic Acid: 3-Oxoacyl-Coenzyme A Thiolase as the Possible Site of Inhibition

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Inhibition of fatty acid oxidation by metabolites of hypoglycin (von Holt *et al.*, 1966) and by its simple analogue pent-4-enoic acid (Sherratt *et al.*, 1971) could be due to sequestration of CoA or to inhibition of a specific enzyme (McKerns *et al.*, 1960). Our work (Sherratt *et al.*, 1971; Holland & Sherratt, 1971) points to inhibition of β -oxidation, and a search was made for the site. The use of enzymes from ox and pig tissues was justified, since pent-4-enoic acid inhibits fatty acid oxidation by mitochondria from these sources. CoA esters of various fatty acids were tested as inhibitors (see Sherratt *et al.*, 1971); pent-4-enoyl-CoA, penta-2,4-dienoyl-CoA and acryloyl-CoA are metabolites of pent-4-enoic acid, and *n*-pentanoyl-CoA, cyclopropanecarbonyl-CoA and cyclobutanecarbonyl-CoA were used as controls.

No significant inhibitions of purified acyl-CoA dehydrogenases (EC 1.3.99.2 or EC 1.3.99.3), enoyl-CoA hydratase (EC 4.2.1.17) or 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) by any acyl-CoA were