Arnow (1937) test: this was probably 3-fluorocatechol (Goldman, Milne & Pignataro, 1967). Fluoride accumulated and ultimately accounted for about 85% of the fluorine originally present in the *o*-fluorobenzoate. Fluoromuconate was detected in small amounts. Massive amounts of Arnow-positive material were found during growth in the presence of *m*-fluorobenzoate: some of this disappeared as the brown condensation products accumulated. About 45% of the fluorine was recovered as fluoride and some fluoromuconate was also formed. Metabolism of *p*-fluorobenzoate during growth on benzoate led to the accumulation of fluoride, but fluorocatechol and fluoromuconate were formed in only small and transitory amounts.

The metabolism of these fluoro-substituted compounds was discussed with reference to the known mechanism of oxidation of benzoate, and also to the degradation of fluorobenzoates in other organisms (e.g. Hughes, 1965; Goldman *et al.* 1967; Smith, Tranter & Cain, 1968; Cain, Tranter & Darrah, 1968).

A. L. is in receipt of Science Research Council Studentship.

Ali, D. A., Callely, A. G. & Hayes, M. (1962). *Nature, Lond.*, **196**, 194.

Arnow, L. E. (1937). *J. biol. Chem.* **118**, 531.

Cain, R. B., Tranter, E. K. & Darrah, J. A. (1968). *Biochem. J.* **106**, 211.

Callely, A. G. & Jones, J. G. (1965). *Biochem. J.* **97**, 11c.

Goldman, P., Milne, G. W. A. & Pignataro, M. T. (1967). *Arch. Biochem. Biophys.* **118**, 178.

Hughes, D. E. (1965). *Biochem. J.* **96**, 181.

Kennedy, S. I. T. & Fewson, C. A. (1968). *J. gen. Microbiol.* (in the Press).

Smith, A., Tranter, E. K. & Cain, R. B. (1968). *Biochem. J.* **106**, 203.

The Metabolism of 2-Furoic Acid by *Pseudomonas fluorescens* F2

By P. W. TRUDGILL (*Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth*)

The direct conversion of the carbon skeleton of 2-furoate into 2-oxoglutarate by whole cells of 2-furoate-grown *Pseudomonas fluorescens* F2 has recently been reported (Jones & Trudgill, 1967). This report describes work carried out with cell-free systems.

Disruption of bacteria in the French pressure cell followed by centrifuging at 27000*g* for 10 min. yielded a turbid supernatant capable of oxidizing 2-furoate with the consumption of 2.5 moles of O₂, at a rate of 4 μ l./hr./mg. of protein, for each mole of

2-furoate utilized. Activity was enhanced fourfold in the presence of 1 mM-methylene blue, but 2,6-dichlorophenol-indophenol, phenazine methosulphate, FMN and FAD were inactive.

Further fractionation of the low-speed supernatant at 200000*g* for 60 min. yielded an inactive supernatant that could be activated by addition of the resuspended pellet or by methylene blue, suggesting that soluble enzymes involved in metabolic transformations of 2-furoate have an obligatory requirement for a suitable electron-transport system.

Dialysed low-speed supernatant was inactive towards 2-furoate even in the presence of methylene blue, but could be reactivated by the addition of ATP, CoA and NAD. The utilization of ATP and CoA for CoA ester formation was demonstrated by the inhibition of oxygen uptake and the accumulation of 2-furoate hydroxamate that occurred in the presence of hydroxylamine. The endogenous activity of such a system was negligible, and methylene blue reduction accompanying furoate utilization could be followed spectrophotometrically under anaerobic conditions. Reduction of methylene blue was not inhibited by 2 mM-sodium azide, 4 mM-EDTA or 4 mM- α '-bipyridyl, but was 50% inhibited by 2 mM-potassium cyanide.

Fractionation of the low-speed supernatant with ammonium sulphate resulted in 80% of the activity being recovered in the 0-35%-saturated fraction. This oxidized 1 mole of 2-furoate in the presence of ATP, CoA, NAD and methylene blue with the consumption of 0.45 mole of O₂ and the accumulation of an unidentified keto acid, which formed a DNP-hydrazone giving a stable colour in alkali, λ_{max} 471 m μ .

Though the stoichiometry of this system, its requirement for an electron acceptor and independence of molecular oxygen are characteristics similar to those described for the hydroxylation of nicotinic acid (Hunt, Hughes & Lowenstein, 1958) and picolinic acid (Dagley & Johnson, 1963) in which the incorporated oxygen is derived from water, such a reaction has not been conclusively demonstrated. If hydroxylation does occur, the prior requirement for CoA ester formation is a somewhat unusual feature.

Dagley, S. & Johnson, P. A. (1963). *Biochim. biophys. Acta*, **78**, 577.

Hunt, A. L., Hughes, D. E. & Lowenstein, J. M. (1958). *Biochem. J.* **69**, 170.

Jones, A. H. & Trudgill, P. W. (1967). *Biochem. J.* **105**, 31P.