We therefore propose the photometabolism of benzoate by *Rhodopseudomonas palustris* occurs by the following pathway:

Benzoate $\rightarrow$ hydrogenated benzoate derivatives $\rightarrow$ cyclohexanol-2-carboxylate $\rightarrow$ [cyclohexanone-2-carboxylate] $\rightarrow$ pimelate.

Further metabolism may be accomplished by β -oxidation.

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The Extracellular Agarase from a *Cytophaga* Species

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A *Cytophaga* species (N.C.M.B. 1327), previously shown to produce intracellular enzymes that cause breakdown of the polysaccharides agarose and porphyran (Turvey & Christison, 1967*a,b*), was used as the source of an extracellular enzyme with similar activity. When the bacterial cells were removed from cultures at the exponential phase of growth, the cell-free supernatant solution had enzyme activity. The enzyme was isolated by precipitation with ammonium sulphate followed by chromatography on DEAE-Sephadex A-50 (OH⁻ form). The latter step was necessary to free the enzyme preparation from associated polysaccharides.

The purified enzyme differed from that obtained by ultrasonic disintegration of the bacterial cells in that it appeared to be a single enzyme. The ratio of its activity on porphyran measured viscometrically to that measured by production of reducing power (Turvey & Christison, 1967*a,b*) was 25:1 compared with the corresponding ratio 1:9 found for the intracellular enzyme preparation. The extracellular enzyme is thus seen to be an endo-enzyme, producing rapid depolymerization of the substrate without production of low-molecular-weight sugars. This type of activity was confirmed by studying the products of its action on porphyran. The major products were oligosaccharides with only small amounts of the disaccharide, neoagarobiose, and no monosaccharides, compared with the action of the intracellular enzyme, which produced some monosaccharides.

The enzyme displays greatest activity to (a) agarose, with decreasing activity to (b) alkali-treated porphyran (Rees, 1961), (c) porphyran, (d) the

alkali-treated galactan sulphate of *Laurencia pinnatifida*, and (e) the native galactan sulphate of *L. pinnatifida* (Bowker & Turvey, 1968*a,b*), in that order. This is consistent with a specificity for substrates containing an alternating sequence of 3-linked D-galactose and 4-linked 3,6-anhydro-L-galactose units, as in agarose. Increasing departure from this type of structure results in lowering of the extent of enzyme action.

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Metabolism of Monofluorobenzoates by Bacterium N.C.I.B. 8250

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Ali, Calley & Hayes (1962) showed that bacterium N.C.I.B. 8250 oxidized *m*- and *p*-fluorobenzoate after growth on benzoate. Calley & Jones (1965) subsequently demonstrated that *o*- and *p*-fluorobenzoate, and possibly *m*-fluorobenzoate, were inducers for the benzoate-oxidizing system in this organism. We have confirmed these observations in the course of investigations into the metabolism of aromatic compounds by bacterium N.C.I.B. 8250 (Kennedy & Fewson, 1968) and have now examined the metabolic fates of the monofluorobenzoates.

None of the monofluorobenzoates was utilized as sole source of carbon and energy for growth of bacterium N.C.I.B. 8250. Cell yield on limiting benzoate, salicylate or succinate, however, was increased by suitable concentrations of *o*-fluorobenzoate. Yield in the presence of *m*-fluorobenzoate was slightly augmented but the appearance of brown material in the medium made estimates of growth difficult. *p*-Fluorobenzoate generally decreased the yield on other carbon sources but was itself metabolized. Both *o*-fluorobenzoate and, to a smaller extent, *m*-fluorobenzoate appeared to be incorporated into cell material since the specific radioactivity of cells grown on [*ring*-U-¹⁴C]-benzoate was decreased in the presence of these two compounds.

Examination of the medium during growth on benzoate + *o*-fluorobenzoate showed the transitory appearance of a compound reacting positively in the

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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).

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

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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.

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