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Benzoate Oxidation by Bacterium N.C.I.B. 8250

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Although Ichihara, Adachi, Hosokawa & Takeda (1962) described a cell-free system for benzoate oxidation in *Pseudomonas aeruginosa* and *Micrococcus ureae*, several workers have experienced difficulties in measuring benzoate oxidase activity in bacteria and have had to resort to experiments with intact organisms (e.g. Mandelstam & Jacoby, 1965; Kemp & Hegeman, 1968; Kennedy & Fewson, 1968).

We have developed a specific, rapid and sensitive method for measuring benzoate consumption by whole cells or by extracts. This is based on u.v.-absorption measurements of residual benzoic acid after acidification of reaction mixtures and extraction through chloroform. It has several advantages over previous methods, which have generally depended on measurement of oxygen consumption with consequent uncertain corrections for oxygen uptake caused by endogenous metabolism, NADH oxidation or metabolism of the products of benzoate oxidation.

Whole cells of bacterium N.C.I.B. 8250 grown in a benzoate-salts medium oxidise benzoate at a rate of about 70nmol/min per mg of protein. After ultrasonic disruption and centrifugation at 10000g for 20min the supernatants show a low but reproducible benzoate oxidase activity (about 2nmol/min per mg of protein). Optimum activity is obtained by extraction and assay in tris buffer, pH 7.5. The enzymic activity has a specific requirement for Fe²⁺ ions. Preincubation with cupferron (30μM) or 2,2'-bipyridyl (100μM), but not sodium diethyldithiocarbamate (30μM), prevents benzoate oxidation. There is a requirement for FAD (5μM). Enzymic activity also depends on the continuous regeneration of NADH (ethanol and alcohol-NAD oxidoreductase, EC 1.1.1.1). NADPH is not effective. Benzoate does not disappear under anaerobic conditions. Various combinations of ATP, CoA, thiamin pyrophosphate, lipoic acid, Mg²⁺, mercaptoethanol and GSH have no beneficial effect on the rate of benzoate oxidation. A compound giving a positive Rothera reaction is formed as benzoate disappears from the reaction mixture. Presumably the active catechol oxygenase (EC 1.13.1.1) (Kennedy & Fewson, 1968) accounts for the absence of catechol accumulation.

The addition of extracts of bacterium N.C.I.B. 8250 inhibits benzoate oxidation by intact cells. This is superficially reminiscent of the situation in *Rhodopseudomonas palustris* (Dutton & Evans, 1968), but it is unlikely that the mechanism of inhibition is the same since the pathways of benzoate metabolism are quite different in the two organisms.

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Metabolism of Myricetin, Myricitrin and 3,4,5-Trihydroxyphenylacetic Acid

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Previous studies on the metabolism of flavonoid compounds in mammals have largely been restricted to flavonoids showing hydroxylation in the 4'- or 3', 4'-positions only in the B ring, although flavonoids possessing a 3',4',5'-trihydroxyphenyl grouping are known to occur in plants of dietary importance.

Administration of myricetin (3,5,7,3',4',5'-hexahydroxyflavone) (200mg) to rats maintained on our standard diet gave rise to a major metabolite in the urine that has been identified as 3,5-dihydroxyphenylacetic acid by chromatographic and spectral methods. No glucuronide conjugates of myricetin were detected.

Since earlier studies have shown that the degradation of flavonoids is in part mediated by intestinal micro-organisms (Griffiths, 1964; Nakagawa, Shetlar & Wender, 1965; Das & Griffiths, 1968), the ability of intestinal micro-organisms to degrade myricetin *in vitro* was studied by incubating myricetin in a thioglycollate broth with mixed inocula of micro-organisms obtained by sterile section of the rat caecum. The incubation was carried out under anaerobic conditions at 37°C. The metabolites were recovered by a modification of a previously described ether extraction procedure (Griffiths, 1964) and shown to be 3,5-dihydroxyphenylacetic acid, 3,4,5-trihydroxyphenylacetic acid and unchanged myricetin.

Since it appeared probable that 3,4,5-trihydroxyphenylacetic acid was an intermediate in the