phenyls are used in the plastics industry, and their persistence in the environment is giving cause for concern; it may be possible to select those with the desired properties and still be biodegradable.

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Cinnamic Acid and *p*-Coumaric Acid, Precursors of Ubiquinone in Higher Plants, Green Algae and Fungi

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Previously we have reported on the incorporation of *p*-hydroxybenzoic acid into ubiquinone by the higher plants maize and French bean, the green algae *Euglena gracilis* and *Ochromonas danica*, the moulds *Agaricus campestris* and *Phycomyces blakesleeanus*, and the yeast *Saccharomyces cerevisiae* (Whistance, Threlfall & Goodwin, 1967; Spiller, Threlfall & Whistance, 1968; Threlfall & Whistance, 1970). We now report on the incorporation into ubiquinone by maize, *E. gracilis*, *P. blakesleeanus* and baker's yeast of cinnamic acid and *p*-coumaric acid, compounds known to give rise to *p*-hydroxybenzoic acid in higher plants (Zenk, 1966) and some fungi (Yoshida, 1969).

Maize shoots (200), *E. gracilis* cells (20g wet wt.), baker's yeast cells (100g wet wt.) and *P. blakesleeanus* mycelia (35g wet wt.) were incubated for 18h (maize) or 6h (*E. gracilis*, baker's yeast and *P. blakesleeanus*) under our standard conditions (Whistance *et al.* 1967; Spiller *et al.* 1968; Threlfall & Whistance, 1970) with either 4 μ Ci of [14 C]cinnamic acid (10mCi/mmol) or 4 μ Ci of *p*-[14 C]coumaric acid (12.9mCi/mmol) [prepared by incubating L-[14 C]phenylalanine and L-[14 C]tyrosine respectively with phenylalanine ammonia-lyase from *Rhodotorula glutinis* 1FO 559 (Ogata, Uchiyama, Yamada & Tochikura, 1967)]. At the end of the incubations the ubiquinones were isolated from the organisms and assayed for radioactivity (Whistance *et al.* 1967; Spiller *et al.* 1968; Threlfall & Whistance, 1970).

In all cases radioactivity was incorporated into ubiquinone, the major radioactive compound observed in the lipid fractions. The incorporation (percentage of dose) from [14 C]cinnamic acid varied from 0.008 in maize to 0.0005 in baker's yeast; that from *p*-[14 C]coumaric acid varied from 0.11 in baker's yeast to 0.03 in maize. The specific radioactivities (c.p.m./ μ mol) of the samples

labelled from [14 C]cinnamic acid were 1860, 1228, 293 and 12 for maize, *E. gracilis*, *P. blakesleeanus* and baker's yeast respectively; those of the samples labelled from *p*-[14 C]coumaric acid were 4450, 5310, 2630 and 800 respectively. Ozonolytic degradations and comparison of the specific radioactivities of the ubiquinone samples with those of the 4-demethylsterols established that greater than 90% of the radioactivity in the ubiquinone molecules was in the *p*-benzoquinone ring.

The results show that in all organisms examined the *p*-hydroxybenzoic acid utilized for ubiquinone biosynthesis can be formed from *p*-coumaric acid and, with the possible exception of yeast, cinnamic acid. However, they do not rule out the possibility that some of this *p*-hydroxybenzoic acid is also formed directly from chorismic acid.

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Oxidation of Furan-2-Carboxylate to 2-Oxoglutarate by *Pseudomonas putida* F2: Studies of Enzymology and Electron Transport

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Pseudomonas putida F2 grown with furan-2-carboxylate oxidizes this compound to 2-oxoglutarate through the following sequence of intermediates: furan-2-carboxylate $\rightarrow$ 2-furoyl-CoA $\rightarrow$ 5-hydroxy-2-furoyl-CoA $\rightarrow$ 5-oxo- Δ^2 -dihydro-2-furoyl-CoA $\rightarrow$ 2-oxoglutaryl-CoA $\rightarrow$ 2-oxoglutarate. A key reaction of this sequence, the hydroxylation of 2-furoyl-CoA, draws its oxygen from water with the resultant obligatory requirement for an electron acceptor, which may be satisfied by Methylene Blue or the membrane fraction from furan-2-carboxylate-grown cells (Jones & Trudgill, 1967; Trudgill, 1969).

Descriptions of other hydroxylations that draw their oxygen from water are confined to nitrogen-containing hydrophilic ring structures (Dagley &

Johnson, 1963; Hunt, Hughes & Lowenstein, 1958; Hayaishi, 1969). A similar hydrophilic substrate requirement perhaps dictates the formation of 2-furoyl-CoA as substrate for hydroxylation by *P. putida* F2.

The soluble enzyme system that catalyses the conversion of furan-2-carboxylate into 2-oxoglutarate has been purified 30-fold by $(\text{NH}_4)_2\text{SO}_4$ fractionation (0–31% saturation fraction), dialysis, protamine sulphate treatment and chromatography on Sephadex G-200, which separates the system into two fractions. The first fraction (mol.wt. about 200 000) contains the 2-furoyl-CoA hydroxylase, which can be assayed with synthetic 2-furoyl-CoA as substrate and Methylene Blue as electron acceptor in an anaerobic cuvette. The second fraction (mol.wt. about 100 000) contains the 2-furoyl-CoA synthetase and the 5-oxo- Δ^2 -dihydro-2-furoyl-CoA (lactone) hydrolase. We have not yet ascertained which fraction contains the 2-oxoglutaryl-CoA hydrolase or whether the two obligatory keto-enol tautomerizations are assisted by the agency of enzymes.

Spheroplasts (Repaske, 1956) prepared from furan-2-carboxylate-grown cells yield membrane preparations that facilitate a CO- and KCN (1 mM)-sensitive transport of electrons from the 2-furoyl-CoA hydroxylase reaction to oxygen. Membranes prepared under identical conditions from glutamate- or nutrient-broth-grown cells are quite inactive in this capacity. Reduced minus oxidized cytochrome difference spectra show the presence of cytochromes a_1 , b_1 and c-551; neither they nor the ubiquinone and flavin content of membrane fractions show detectable variations consistent with change of growth substrate. Although crucial differences may be masked in crude preparations, the apparent requirement for a furan-2-carboxylate-induced membrane-bound component, specifically required if electrons from the 2-furoyl-CoA hydroxylase are to be accepted, is an interesting phenomenon.

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Some Biochemical Studies on Warfarin Resistance in the Rat

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Warfarin [3-(α -acetylbenzyl)-4-hydroxy-coumarin] has been used as an anticoagulant rodenticide for over 20 years. The first reported case of failure to obtain field control of rats (*Rattus norvegicus* L.) due to warfarin resistance was reported from Scotland by Boyle (1960). Genetic selection in this laboratory has resulted in the isolation of a strain of rat that is homozygous for warfarin resistance of Scottish origin (Greaves, personal communication). Ikeda, Conney & Burns (1968) have shown that warfarin is metabolized by a microsomal enzyme capable of induction by phenobarbitone, chlordane or DDT. We have investigated the possibility that resistance may be associated with increased microsomal enzyme activity.

A comparative study has been made of the liver drug-metabolizing enzyme systems of susceptible and resistant male rats. Their activities *in vivo* were assessed by comparing the 'sleeping times' of animals receiving an intraperitoneal injection of 30 mg of pentobarbitone sodium/kg body wt. The warfarin-resistant rats showed a significantly shorter sleeping time. The aromatic hydroxylation of aniline *in vitro* was assayed in postmitochondrial supernatants by using a modification of the method described by Kato & Gillette (1965). The liver microsomal cytochrome P-450 concentrations were determined by a method based on that of Omura & Sato (1964). The concentrations of both systems were significantly higher in the resistant strain. Other differences were noticed in the relative liver weight of both resistant and susceptible animals. Microsomal enzyme activities were affected to a different extent in both strains when DDE was incorporated in the diet.

Warfarin detoxication in susceptible rats has been shown to involve several metabolites, including four hydroxylated products (Barker, Hermodson & Link, 1969). Although large interstrain differences have been demonstrated with microsomal drug metabolism in four laboratory rat strains (Furner, Gram & Stitzel, 1969), it is possible that warfarin resistance in this species may involve a greater or more rapid metabolism of this compound.

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