Catabolism of 1-Phenylalkanes by *Nocardia salmonicolor* (N.C.I.B. 9701)

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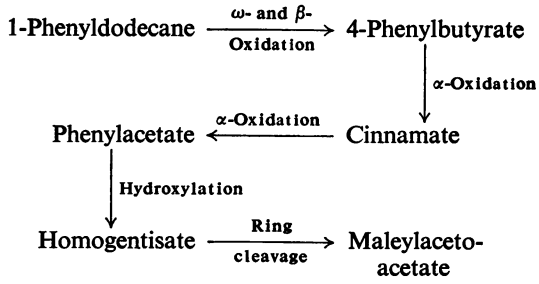
Previous studies demonstrated that *Nocardia* isolates from soil utilize 1-phenylalkanes as sole carbon and energy sources for growth (Webley *et al.*, 1955, 1956; Davis & Raymond, 1961). Baggi *et al.* (1972) have since described the metabolism of 2-, 3- and 4-phenylalkanes by *Pseudomonas* and *Nocardia* isolates.

The present communication reports that *Nocardia salmonicolor* (N.C.I.B. 9701) grows on various *n*-alkanes, 1-phenylalkanes and simple aromatic compounds as sole carbon and energy sources. The organism grows readily in stirred fermenters at 30°C with 1-phenylnonane or 1-phenyldodecane as substrate. When medium containing 0.5% (v/v) of 1-phenyldodecane was inoculated with washed nutrient-broth-grown organisms there was a 6h lag phase during which rapid induction of isocitrate lyase (EC 4.1.3.1) occurred. Exponential growth followed (mean generation time 6h) and isocitrate lyase activity reached a maximum value of 100nmol·min^{-1}·(mg of protein)$^{-1}$. There was a marked diauxie (lag 20h) and isocitrate lyase activity was decreased towards the end of the first growth phase, being further lowered during the ensuing lag and second growth phase.

During the first growth phase 4-phenylbutyrate, 4-phenybut-3-enoate and cinnamate accumulated in the medium, 4-phenylbutyrate being the first to appear. Approx. 14% of the growth substrate accumulated as these acids, which disappeared from the medium at the onset of diauxic lag, when there was a concomitant accumulation of four monohydroxylated phenolic acids, one being identified as 4-(*o*-hydroxyphenyl)butyrate and another as a 4-hydroxyphenylbut-3-enoate. These hydroxylated compounds were utilized during the second, shorter, growth phase. Additionally, phenylacetate was isolated from culture media after growth under O_2-limited conditions.

1-Phenyldodecane-grown organisms rapidly oxidized 4-phenylbutyrate, cinnamate, phenylacetate and homogentisate, but not benzoate or protocatechuate, and cell-free extracts contained homogentisate oxygenase activity (Chapman & Dagley, 1962). These results suggest that 1-phenyldodecane is metabolized as follows:



Evidence will be presented that accumulation of hydroxylated compounds reflects metabolic imbalance resulting from exhaustion of the growth substrate and consequent diminution in the supply of acetate units from the side chain.

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Inhibition Studies on the Aromatization of Androst-4-ene-3,17-dione by Human Placental Microsomal Preparations

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The nature of the mixed-function oxidases present in placentae has recently aroused much attention. Cytochrome *P*-450 has been reported to be present in low concentrations in the mitochondrial and microsomal fractions of placentae of different species, and activities of drug-metabolizing enzymes are correspondingly low (Meigs & Ryan, 1968; Chakraborty *et al.*, 1971). However, the placenta is capable of synthesizing many steroids, especially oestrogens, and aromatization of androgens to oestrogens occurs in most species except rabbits and guinea pigs (Hagerman, 1969).

Using a radiochemical method for the determination of the rate of conversion of [7α-^{3}H]androst-4-ene-3,17-dione into [7α-^{3}H]oestrone, we have studied the nature of the steroid-ring-A-aromatizing enzyme present in a stored microsomal fraction of full-term human placentae. The enzyme system requires NADPH and O_2 (Akhtar & Skinner, 1968), is not affected by CO and no marked loss of activity occurs

on conditions of storage that degrade cytochrome *P*-450 to cytochrome *P*-420. As expected for a membrane-bound enzyme complex, it has proved resistant to solubilizing techniques (sonication, detergents, deoxycholate, trypsin), and treatment with deoxycholate, steapsin or papain markedly decreases aromatizing activity. Inhibition studies with classical inhibitors (0.1mM) of hepatic microsomal drug-hydroxylating enzymes [SKF 525A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride), DPEA (2,4 - dichloro - 6 - phenylphenoxethylamine), Lilly 18947 (2,4 - dichloro - 6 - phenylphenoxyethyldiethylamine), ethyl isocyanide], competitive inhibitors of these enzymes {imipramine [10,11-dihydro-5-(3-dimethylaminopropyl)dibenz-[*b*,*f*]azepine], biphenyl, zoxazolamine (2-amino-5-chlorobenzoxazole), DDT [1,1,1 - trichloro - 2,2 - bis - (*p* - chlorophenyl)ethane], dieldrin (1,2,3,4,10,10 - hexachloro - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene)}, inhibitors of steroid hydroxylation {spironolactone [β-(7α-acetylthio-17β-hydroxy-3-oxoandrost-4-en-17α-yl)propionic acid lactone], betamethasone (9α-fluoro-11β,17α,21 - trihydroxy - 16β-methylpregna - 1,4 - diene -3,20 - dione), metyrapone [2-methyl-1,2-bis-(3-pyridyl)propan-1-one]} and chelating agents (EDTA, αα′-bipyridyl) were without effect on the aromatizing system. However, inhibition did occur at this concentration (0.1mM) with testosterone (90%), [α-(*p*-aminophenyl)-α-ethylglutarimide] aminoglutethimide (60%), an inhibitor of desmolase, and with the thiol-blocking reagents *p*-hydroxy-mercuribenzoate (80% inhibition) and iodoacetamide (20%), but not with β-mercaptoethylamine. Slight inhibition also occurred at 0.1mM with the fatty acids arachidonic acid (30%) and lauric acid (20%). It has been postulated that metyrapone inhibits steroid and drug hydroxylations by interaction with cytochrome *P*-450 (Hildebrandt *et al.*, 1969). We interpret this lack of inhibitory activity on the aromatizing enzyme, together with the absence of effect by CO and ethylisocyanide, as conclusive evidence that the conversion of androgens into oestrogens in the placenta is not mediated by cytochrome *P*-450.

These results from experiments *in vitro* indicate that, although the placenta has a poor ability to metabolize drugs, the presence of drugs and other xenobiotics in the placenta is unlikely to adversely affect the metabolism of androgens to oestrogens.

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The Deoxyfluoro-D-glucopyranose 6-Phosphates and their Effect on Yeast Glucose Phosphate Isomerase

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Glucose phosphate isomerase (EC 5.3.1.9) catalyses the isomerization of D-glucose 6-phosphate into D-fructose 6-phosphate. D-Glucose 6-phosphate binds in its α-pyranose form (Salas *et al.*, 1965) and is then converted into its acyclic form (Dyson & Noltmann, 1968), probably by a concerted ring-opening mechanism. Sugar phosphates that can only exist in an acyclic form (e.g. D-erythrose 4-phosphate and D-gluconic acid 6-phosphate) are good competitive inhibitors.

The deoxyfluoro-D-glucopyranose 6-phosphates were prepared from the corresponding deoxyfluoro-D-glucose and ATP by using hexokinase. 2-Deoxy-2-fluoro-, 3-deoxy-3-fluoro- and 4-deoxy-4-fluoro-D-glucose 6-phosphate were thus prepared. In addition, 2-chloro-2-deoxy-D-glucose 6-phosphate was obtained from 2-chloro-2-deoxy-D-glucose by this method.

Glucose phosphate isomerase was assayed by coupling to the phosphofructokinase reaction. The rate of proton release was followed spectrophotometrically by using Cresol Red. This method can provide a sensitive and convenient assay system. Both 3-deoxy-3-fluoro and 4-deoxy-4-fluoro-D-glucose 6-phosphate were substrates for glucose phosphate isomerase and, in addition, the products of this reaction, 3-deoxy-3-fluoro- and 4-deoxy-4-fluoro-D-fructose 6-phosphate respectively, were very good substrates for phosphofructokinase. The K_m values of the 3-deoxy-3-fluoro- and the 4-deoxy-4-fluoro-D-glucose derivatives for glucose phosphate isomerase were 6 and 7mM respectively. The $V_{max.}$ values relative to D-glucose 6-phosphate were 0.006 and 0.31 respectively. The low relative $V_{max.}$ values for the 3-deoxy-3-fluoro derivative are not explained by the inductive effect of the fluorine substituent, since this should facilitate rather than hinder the formation of the proposed enediol intermediate. Although it appears that the 3-hydroxyl group in D-glucose 6-phosphate is not of vital importance in the binding of the substrate to the enzyme, it might be important in