and tea intake. Drug ingestion also had no significant effect on monooxygenase function which was not unexpected as only one patient was receiving a known enzyme inducer. Cigarette smoking significantly increased AHH activity ($P < 0.05$) and cytochrome P-450 content when expressed per g/liver but not per mg microsomal protein but had no effect on NADPH cytochrome c reductase activity. In the smokers, there was no relationship between number of cigarettes smoked and the degree of AHH induction.

We have thus been able to show significant correlations between two environmental factors and *in vitro* monooxygenase activity in microsomal fractions of human liver.

ARB is an MRC Fellow

**Reference**

BOOBIS, A.R., BRODIE, M.J., DAVIES, D.S., FLETCHER, D.H. & SAUNDERS, J.H. (1978). Mixed function oxidase activity in microsomal fractions of human liver biopsy specimens. *Br. J. clin. Pharmac.*, **6**, 449P–450P.

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## Resolution and reconstitution of two forms of hepatic cytochrome P-450 from rabbits

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As part of a programme of investigation into the substrate specificity of cytochrome P-450 we have attempted to resolve individual forms of this haemoprotein from rabbit liver microsomes. Resolution of cytochrome P-450 and NADPH-cytochrome P-450 reductase was performed essentially as described by Johnson & Muller-Eberhard (1977). Solubilisation of the microsomal fraction with sodium cholate was followed by polyethylene glycol fractionation. Treatment of the precipitated material with Renex, a non-ionic detergent and chromatography on a hydroxylapatite-cellulose column enabled two fractions of cytochrome P-450 to be obtained. Detergent was removed by dialysis followed by adsorption on to calcium phosphate gel. NADPH-cytochrome P-450 reductase was purified by chromatography of the 25–60% ammonium sulphate precipitate of the supernatant of the appropriate polyethylene glycol fraction on DEAE-cellulose followed by adsorption on to calcium phosphate gel and concentration by ultra-filtration.

Two forms of cytochrome P-450 were partially purified with specific contents of up to 9.8 nmol/mg protein. SDS polyacrylamide gel electrophoresis revealed that one fraction (fraction 1) contained only a single band, molecular wt 54,000, whereas the second fraction (fraction 2) comprised several proteins, molecular weights between 45,000 and 60,000. Treatment of rabbits with 3-methylcholanthrene (80 mg/kg in corn oil injected intraperitoneally) increased fraction I whereas treatment of rabbits with phenobarbitone (1 mg/ml in drinking water for 4 days) increased fraction II. Fraction I had an absorption maximum of the carbon monoxide complex at 448 mm whereas the absorption maximum of fraction II was 450 nm.

Both fractions of cytochrome P-450 could be reconstituted to give aryl hydrocarbon hydroxylase and 7-ethoxycoumarin 0-deethylase activity. Activity was dependent on the presence of both cytochrome P-450 and the reductase fraction and was enhanced by the addition of lipid.

Fraction I from methylcholanthrene treated rabbits was more active than fraction I from phenobarbitone-treated or control animals in supporting aryl hydrocarbon hydroxylase activity (375 pmol $nmol^{-1}$ $min^{-1}$). Fraction II from phenobarbitone-treated animals was least active (86 pmol $nmol^{-1}$ $min^{-1}$). Fraction I from phenobarbitone-treated animals was most active in supporting 7-ethoxycoumarin 0-deethylase activity.

α-Naphthoflavone (500 μM) a selective inhibitor of monooxygenase activity, inhibited aryl hydrocarbon hydroxylase activity with fraction I from methylcholanthrene-treated animals by over 70% and from phenobarbitone-treated animals by 50%. With fraction II from both groups of animals 2 μM α-naphthoflavone inhibited aryl hydrocarbon hydroxylase activity by 50–80% but at higher concentrations enhanced activity supporting the suggestion by others that fraction II contains more than one form of cytochrome P-450.

We are currently investigating methods to further purify cytochromes P-450 from the microsomal fraction.

ARB is an MRC Fellow. KML is an MRC Student.

**Reference**

JOHNSON, E.F. & MULLER-EBERHARD, U. (1977). Resolution of two forms of cytochrome P-450 from liver microsomes of rabbits treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. biol. Chem.*, **252**, 2839–2845.