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Kinetic Studies of the N-Demethylation of Ethylmorphine by a Cytochrome P-450-Dependent Enzyme System in Human Liver Microsomes

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Oxidation is an important pathway for the inactivation of many lipid-soluble drugs and other foreign chemicals. Studies with liver microsomes from different species of laboratory animals have established a good inverse correlation between the duration of action of many lipid-soluble drugs *in vivo* and the activity of the drug-oxidizing enzymes of the different species (Quinn, Axelrod & Brodie, 1958). However, there have only been a few studies of human liver microsomal enzymes and in many cases the liver samples were obtained either at post mortem or were kept for periods of 1–3 h before use (Ackerman, 1970; Alvares *et al.* 1970). In view of the important role these enzymes may play in determining individual differences in response to various drugs in man (Davies, 1969) it was important to make kinetic studies of the oxidation of drugs with fresh samples of human liver.

Samples of liver were taken during abdominal surgery with the informed consent of the patient. Isolation of the microsomal fraction was commenced within 5–10 min of the samples being taken.

Concentrations of microsomal protein per g of liver ranged from 9.5 to 23 mg in the ten samples analysed. The concentration of cytochrome P-450 ranged from 0.67 to 1.02 nmol/mg of microsomal protein. These values are within the range of concentrations of cytochrome P-450 previously found in different species of laboratory animals (Davies, Gigon & Gillette, 1969). The maximal rates ($V_{\max}$) of N-demethylation of ethylmorphine showed a greater than fourfold range (2.0–8.8 nmol/min per mg), the highest value being similar to that found in the adult male rat. The apparent Michaelis constant (K_m) for the N-demethylation of ethylmorphine ranged from 0.8 to 4.0 mM, indicating

that the substrate has a lower affinity for human liver enzymes than for rat enzymes (K_m 0.32 mM). In addition the K_m values increased with $V_{\max}$, as would be the case in a diffusion-limited system (Davies, Gigon & Gillette, 1968).

The rate of reduction of cytochrome c varied from 176 to 282 nmol/min per mg, the equivalent value for the rat being 158 ± 24.3 . The rate of reduction of cytochrome P-450 ranged from 2.76 to 4.29 nmol/min per mg, which is less than in the rat. Reduction of cytochrome P-450, which is thought to be rate-limiting in the oxidation of drugs (Davies, 1969), correlated with $V_{\max}$ values and was increased after the addition of 2 mM-ethylmorphine. However, as has been previously reported (Gigon, Gram & Gillette, 1969), 0.5 mM-aniline decreased the initial rate of reduction of the cytochrome.

These observations demonstrate that there are individual differences in the rates of oxidation of ethylmorphine by cytochrome P-450-dependent enzymes in human liver microsomes. These differences are apparently not due to variations in the concentration of cytochrome P-450, but rather to differing rates of reduction of the cytochrome.

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The Electrophoretic Mobilities of Native and Chemically Modified Human Placental Alkaline Phosphatase Isoenzymes

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At least 15 phenotypes of human placental alkaline phosphatase (EC 3.1.3.1) resulting from the action of nine structural genes have been recognized on the basis of the different electrophoretic mobilities of the enzymes at several pH values (Robson & Harris, 1967). However, the enzyme variants are closely similar in other respects (Thomas, 1970). To attempt to infer some of the structural differences that presumably underlie the differences in electrophoretic behaviour, we have compared the electrophoretic mobilities over a range of pH values of three common placental phosphatase