INDUCED DRUG METABOLISM STUDIES WITH INDOLIC SUBSTRATES

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The oxidative enzymes which metabolise drugs and foreign substances are associated with the endoplasmic reticulum of the parenchymal liver cell. Their physiological role in "detoxication" appears to be related to the change in polarity which they bring about in the case of lipid soluble compounds before these can be eliminated through the renal tubule (Brodie, 1964). Ring hydroxylation of aromatic molecules is the main mechanism whereby this change in solubility is effected; at the same time hydroxylation renders the compound susceptible to further modification through conjungative processes. The enzyme systems reside in the pellet resulting from high speed centrifugation (100,000 × g) of the mitochondrial supernatant of liver homogenates. This pellet which constitutes the microsomes consists of membranes with or without ribosomes; the oxidative activity is associated with the smooth membranes (Ernster *et al.*, 1962).

The effect of treating experimental animals with various drugs on the yield of oxidation products of other pharmacologically active substances has been reviewed (e.g. Conney and Burns, 1962). This paper will summarise the effect of pretreatment on indole metabolism with particular reference to lipid soluble tryptamines which are 6-hydroxylated (Jepson *et al.*, 1962) by a liver microsomal system requiring reduced nicotinamide adenine dinucleotide phosphate and molecular oxygen in common with the drug metabolising enzymes (Jaccarini, 1966).

Methods

Wistar albino rats and English white rabbits were injected intraperitoneally daily with inducing agent in a suitable solvent. Controls were given solvent only. The animals were kept on normal diets with free access to water. After the last injection they were starved for 24 hours and killed. Microsomes were isolated according to Mitoma *et al.* (1956), and incubated with substrate in the presence of NADP, nicotinamide, ATP and Mg ions in pyrophosphate buffer pH 7.2 at 37.5 C for one hour in open vessels. 6-Hydroxy products were estimated through the pink colour (515 millimicron) given by the deproteinised incubate with diazotised sulphanilic acid. For "in vivo" work, the animals were kept singly in cages and the urines were collected after 5 hours and after 24 hours from each injection. Metabolites were identified and their abundance estimated by thin layer chromatographic techniques.

Results and Discussion

All the agents used had a stimulating effect with the exception of ethionine in combination with methylcholanthrene and carbon tetrachloride *(Table I)*. Ethionine is a known inhibitor of protein systhesis through its effect on liver ATP (Villa-Trevino *et al.*, 1963). Carbon tetrachloride in dosage as used by Cameron and Karunaratne (1936) destroyed all the hydroxylating capacity with respect to diethyltryptamine.

Normally poor substrates for 6-hydroxylation such as acetyltryptophan were the ones which suffered the greatest stimulation with phenobarbitone. The same effect was obtained in the case of the carcinogenic hydrocarbons, benzpyrene and methylcholanthrene; whilst trytamine is normally a poor substrate for rat

TABLE I

The Effect of Pretreatment on "in vitro" Hydroxylation

Pretreatment	*Animal*	*Substrate*	*Percentage Stimulation of Hydroxylation*
Phenobarbitone	Rat	Tryptamine	22
Phenobarbitone	Rat	N, N-diethyltryptamine	18
Phenobarbitone	Rat	N-acetyltryptophan	31
3,4-benzpyrene	Rat	Tryptamine	25
3,4-benzpyrene	Rabbit	Tryptamine	7
3-methylcholanthrene	Rat	N, N-dimethyltryptamine	5
3-methylcholanthrene	Rabbit	N,N-dimethyltryptamine	34
Tryptamine	Rat	Tryptamine	41
Tryptamine	Rabbit	Tryptamine	12
Ethionine and 3-methylcholanthrene	Rat	Tryptamine	0
Testostrone	Rat (female)	N, N-diethyltryptamine	25
Carbon Tetrachloride	Rat	N, N-diethyltryptamine	complete inhibition.

microsomes, it is very active with rabbit microsomes and the opposite effects hold for the alkyltryptamines; *Table I* shows the reciprocal stimulatory effect on the normal species dependent substrate activity. It is possible, therefore, that both phenobarbitone and the polycyclic hydrocarbons improve the penetration of poor substrates to the active site of the enzyme.

The effect of testosterone indicates that some similarity between the interaction of indolic substrates and steroids with the microsomel system might exist especially since phenobarbitone is known to enhance the hydroxylation of testosterone (Conney and Klutch, 1963) and other anabolic steroids.

Both tryptamine and diethyltryptamine *(Table II)* stimulated their own metabolism.

No single mechanism can be postulated for the inducing effect of the various substances used. In many cases liver enlargement occurred accompanied by the proliferation of the smooth endoplasmic reticulum (Orrenius, 1965). However, anabolic steroids do not produce liver enlargement (Fouts, 1963). Moreover, differential enzyme stimulation also occurs (Creaven *et al.*, 1964). The general finding that a drug is capable of stimulating its own metabolism affords a rationalisation of tolerance and sensitivity. It is likely that drug interactions will be explained at the level of microsomal activity and predictions will be made on this basis.

TABLE II

The Effect of Pretreatment on Urinary Hydroxy Metabolites in the Rat

Day	*Treatment*	*Relative Abundance of Hydroxy Metabolites*
1st	N, N-diethyltryptamine	+
2nd	N, N-diethyltryptamine	+
3rd	N, N-diethyltryptamine	+ +
4th	N, N-diethyltryptamine	+ +
1st	3-methylcholantrene	–
2nd	3-methylcholantrene	–
3rd	N, N-diethyltryptamine	+ + + +
4th	N, N-diethyltryptamine	+ + + +
5th	N, N-diethyltryptamine	+

Summary

Stimulation of indole 6-hydroxylation "in vivo" and "in vitro" is effected through the administration of phenobarbitone,

polycyclic carcinogens and 6-hydroxyiase substrate to rats and rabbits.

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