SPECIES VARIATIONS IN DRUG METABOLISM: INVESTIGATIONS ON THE LIVER MICROSOMAL HYDROXYLATION OF TRYPTAMINE AND RELATED COMPOUNDS

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A characteristic feature of the drug metabolising enzymes of the endoplasmic reticulum of the liver cell is the variation of their activity with species and strain (Parke, 1960). Age (Fouts, 1959), and sex (Axelrod, 1956) differences also occur. The results of experiments on laboratory animals are almost invariably utilised in the preliminary assessment of new compounds as potential drugs. However, it is regularly observed that these results vary qualitatively and quantitatively from animal to animal. It is known, for instance, that amphetamine is deaminated in the rabbit but hydroxylated in the rat and dog; cats are deficient in UDP transglucuronylase; and monomethylaminopyrine is dealkylated at a faster rate than the corresponding monoethyl derivative by rabbit liver preparations whereas similar differences are not observed in several other species. It is apparent, therefore, that the extrapolation of results obtained from animal experiments to man is beset with such fundamental difficulties. That a new drug is free from toxic effects in the laboratory animal does not necessarily mean that it will behave similarly in the human; on the other hand, many potentially useful drugs for man must have been and are being discarded at the preclinical preliminary stages because of their observed toxicity to the experimental animal. Investigations on these differences in drug metabolism amongst the various species particularly those nearest to man are therefore of value especially in so far as they better our understanding of the basic factors governing such differences.

Tryptamines are hydroxylated in the 6 position of the indole ring by a specific microsomal enzyme (Jepson *et al.*, 1962), which is one of a number of aromatic hydroxylases known to be associated with the endoplasmic membranes. Species and other related differences in drug metabolism are well illustrated by this particular enzyme.

Methods

Various tryptamines and indoles were incubated with microsomal preparations obtained from different animal species. The methods for the preparation of microsomal fractions from the animal livers, the incubation conditions and the assay for the 6-hydroxy compounds have been described previously (Jaccarini, 1966).

Results and Discussion

The amounts of the 6 hydroxylated products formed when tryptamine, N,N-dimethyltryptamine, N,N-diethyltriptamine and N-acetyltryptophan were incubated with microsomal preparations from various animal livers are shown in Table I. The figures are expressed in u moles hydroxy derivative / g liver (wet weight) / hour and the microsomal preparation was the supernatant of liver homogenates after centrifugation at $10,000 \times g$.

Substrate susceptibility: Tryptamine is most susceptible to 6-hydroxylation in the rabbit, pig, ox and guinea-pig. Fairly

The Hydroxylation of Tryptamines by Liver Microsomes from different species

TABLE I

Animal	*Substrate*			
	Tryptamine	*N,N-Dimethyl-tryptamine*	*N,N-Diethyl-tryptamine*	*N-Acetyl-tryptophan*
Rabbit (7)*a*	3.6*b*	0.65	0.75	0.13
Pig (1)	3.4	—	—	—
Ox (1)	2.8	—	—	—
Hamster (2)	1.8	2.5	1.9	1.1
Guinea-pig (4)	3.1	1.5	0.9	0.06
Rat (32)	0.15	1.5	2.5	0.08
Mouse (6)	0.1	0.15	1.5	0.02
Pigeon (1)	1.6	—	0.5	—
Frog (4)	0			
Fish:		0	0	0
Bogue (*Box-boops* (2)	—*c*	—*c*	0	0
Trigla species (4)	0	0	—	0

a number of animals.

b amount of hydroxylated product (u moles / g tissue / h).

c values too low to be accurately measured.

reasonable yields are also given by hamster and pigeon microsomes. The N,N-dialkyltryptamines exhibit almost equal rates of conversion in all those species which are capable of hydroxylating them. It would appear that this property would make these substances optimal substrates for studies on indole 6-hydroxylase activity among the various species. It is known (Jaccarini, 1966), furthermore, that *alpha-* and *beta-* alkylated tryptamines are hydroxylated at rates comparable with those of the N,N-dialkyltryptamines; thus *alpha*-ethyltryptamine yields 0.18 and 1.2 u moles of 6-hydroxy derivative per g liver per hour with rabbit and rat microsomes respectively. It may be possible to correlate these results with monoamine oxidase activity for it is known (Heinzelman *et al.*, 1960) that *alpha*-methyl- and *alpha*-ethyltryptamine are not oxidatively deaminated *in vitro* by MAO and are in fact potent inhibitors of this enzyme (Greig *et al.*, 1959). Moreover, the N,N-dialkyltryptamines, being tertiary amines, would also be less susceptible to MAO (Randall, 1946). Finally, N-acetyltryptophan is the least susceptible to indole 6-hydroxylase of all the substrates employed in each animal species. A factor which may be responsible for the low hydroxylation rates of this compound is its deacetylation to inactive tryptophan (Jaccarini, unpublished results).

Species variation: Some animals such as the hamster are able to hydroxylate all the substrates at equal rates. Other species such as rabbit, pigeon and guinea-pig hydroxylate the unsubstituted tryptamine better than its alkyl derivatives. On the other hand, in the case of rat and mouse microsomal preparations the alkyl tryptamines appear to be the better substrates.

The complete absence of hydroxylating ability in frog microsomal preparations may, perhaps, be in keeping with the general finding that the microsomal enzymes are only well developed in land animals. Other species such as fish are capable of eliminating foreign substances or xenobiotics through a process of continuous dialysis through the gill membranes against an infinite volume of water (Brodie and Maickel, 1962). Many exceptions to this plausible theory have, however, been reported. Creaven *et al.* (1965) have noted, for instance, that liver microsomal fractions from amphibians hydroxylate coumnrin and diphenyl. The detection of indole 6-hydroxylase activity in

TABLE II

Sex differences in Tryptamine Liver Microsomal 6-Hydroxylation

Species	*Sex*		*Substrate*		
			Tryptamine	*N,N-Diethyl-tryptamine*	*N-Acetyl-tryptophan*
Rabbit	Male	(10)*a*	3.80*b*	0.71	0.12
	Female	(5)	3.54	0.34	0.18
Rat	Male	(21)	0.16	2.81	0.09
	Female	(9)	0.13	2.45	0.08

a number of animals.

b mean yields (u moles / g tissue / h).

the bogue may be worthy of similar note.

Sex differences: Table II shows the existence of definite differences in indole 6-hydroxylase activity among the sexes. Microsomal preparations from male animals produce higher yields of hydroxy products than the corresponding preparations from females. These differences in the rates of metabolism are characteristic of most drug oxidative enzymes associated with the microsomal fractions of liver homogenates; they can be shown to be operative in several instances of sex differences in drug toxicity (Kato *et al.*, 1962). Such sex differences may have a hormonal basis since adrenalectomy, for instance, brings about increased tissue levels of morphine (Way and Adler, 1960).

Age differences: Microsomal preparations from liver of weanling rats are devoid of any hydroxylating ability towards any of the tryptamine substrates. This absence of activity in the young characterises many of the drug metabolising microsomal enzymes and has therefore significant clinical implications. Drug dosage in children and particularly in infants and the new-born should not be calculated from a formula based on the adult dose (Fingl and Woodbury, 1965); clinical experience with each particular therapeutic agent should rather be the guide in our present state of knowledge about these enzymes in the young. Many of these liver microsomal enzymes are of their very nature adaptive enzymes; they are as yet immature in the young especially in the early days of life. Moreover, the evidence which is available shows that the enzymes tend to develop independently of one another and, unless detailed studies are made with a particular drug and its related enzyme, predictions about dosage and toxicity tend to be unreliable. Further complications arise when these studies are made on experimental animals because of the wide variations observed and the inherent difficulties in extrapolative work to the human.

It will be apparent from the above that, though investigations on animals are always useful, techniques must be developed which will permit direct studies with human tissues; one such technique which is being developed involves the use of post-mortem liver tissue (Jaccarini and Jepson, 1967).

Summary

The variation of liver microsomal indole 6-hydroxylase activity in dfferent animals exemplifies the species dependence of the drug metabolising enzymes. Age and sex are also factors which have to be considered in studies on drug hydroxylations. Since these species variations render the extrapolation of data from animal to man difficult, a partial but direct approach would involve the use of human liver tissue for the investigations *in vitro* which are necessary in the preliminary stages of the development of new drugs.

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