SPECIES AND SEX DIFFERENCES IN THE KINETIC CONSTANTS FOR THE N-DEMETHYLATION OF ETHYL-MORPHINE BY LIVER MICROSOMES

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It is well-known that liver microsomes from male rats are more active than those of female in the oxidation of drugs and sex steroids (Quinn et al., 1952). Recent evidence indicates that there is a marked sex difference in the Michaelis constant as well as V_{max} for the metabolism of hexobarbital (Remmer, Schenkman and Estabrook) and the N-demethylation of ethyl-morphine by liver microsomes of Sprague-Dawley rats (Davies et al., 1967a). Female rats of this strain have higher K_m and lower V_{max} values than do males. Davies et al. (1967a) concluded that the sex differences in K_m values were not caused by the presence of endogenous activators or competitive inhibitors because the K_m values were not changed by addition of the soluble fraction of liver from either sex. Although it is well-known that there are marked species differences in the metabolism of drugs, there has been no attempt to determine whether the species difference is caused by variations in the apparent K_m or by variations in the amount of enzyme. The present paper shows marked species variations in both the K_m and V_{max} for the metabolism of ethyl-morphine.

Materials and Methods

Animal species: All the experiments were made with well-fed animals

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of the following species: rat (Osborne-Mendel strain, 200-220 g weight); mice (NIH general purpose strain, 25-28 g weight); guinea pig (NIH general purpose strain, 360-400 g weight); rabbit (New Zealand strain 2200-2700 g weight); monkey (African green strain, 2200-2700 g weight).

Enzyme preparations: The livers were homogenized in four volumes of ice cold 1.15% potassium chloride with a teflon-glass homogenizer. The homogenate was centrifuged at 9000 x g for 20 min in a Servall refrigerated centrifuge. The supernatant fraction was then centrifuged one hour at 78,000 x g in a Spinco Model L preparative ultracentrifuge (all g values were calculated for the center of the centrifuge tube). The microsomal pellet was suspended in an ice cold solution consisting of 1.15% potassium chloride and 0.02 M in Tris buffer pH 7.4.

Enzyme assay: The final composition of the incubation mixture was: 50 mM Tris buffer pH 7.4; magnesium chloride 5 mM; sodium isocitrate 8 mM; NADP 0.33 mM; isocitric acid dehydrogenase 0.36 units per ml.; microsomal protein about 2 mg per ml and substrate in appropriate quantities. Nicotinamide was omitted because it inhibits the metabolism of various drugs (Gillette, 1966) by a competitive mechanism (Schenkman and Estabrook, 1967).

Ten different concentrations of substrate were used, ranging from approximately 0.3 to 3.0 times the K_m value. The mixtures were incubated for ten minutes at 37° in a Dubnoff shaker. The N-demethylation of ethylmorphine was estimated by measuring the amount of formaldehyde formed according to the method of Nash (Nash, 1953). Under the conditions of the assay the enzymatic reaction was linear with time and protein concentration for all the species tested. For guinea pig and rabbit linearity was observed for 20 min of incubation. The V_{max} and K_m values were calculated with a computer program (Davies et al., 1967b). Results in which the standard error was greater than 15% were not considered valid and thus were omitted.

Protein estimations: Microsomal protein concentrations were determined by the method of Lowry (Lowry et al., 1951).

Results and Discussion

The results obtained are shown in Table I.

TABLE I *

SPECIE	SEX	K_m (mM)	P $\frac{\text{male}}{\text{female}}$	V_{max}**	P $\frac{\text{male}}{\text{female}}$
RAT	MALE	0.30	< 0.001	203	< 0.001
	FEMALE	0.79		42	
MICE	MALE	0.53	< 0.001	139	< 0.001
	FEMALE	1.10		258	
GUINEA PIG	MALE	1.07	> 0.2	67	0.1
	FEMALE	0.96		59	
RABBIT	MALE	1.36	> 0.8	55	0.2
	FEMALE	1.39		64	
MONKEY	MALE	0.86	0.7	93	> 0.4
	FEMALE	0.83		106	

* All values are the mean of at least 8 determinations; except for monkey for which only 5 animals were employed.

** V_{max} is expressed in millimicromoles of formaldehyde formed per milligram of microsomal protein (in 10 minutes at 37°C).

As shown in Table I, there is a marked sex difference in the kinetic constants for the N-demethylation of ethyl-morphine in rats and mice, but no sex difference in guinea pig, rabbit and monkey. The K_m and V_{max} values obtained with the Osborne-Mendel strain of rats closely resembled those of the Sprague-Dawley strain previously reported by Davies, Gigon and Gillette, (1967a).

The results for the mice are particularly interesting because several workers have failed to observe sex differences in drug metabolism in mice (Quinn et al., 1952 and Novick et al., 1966). Dr. E. Vesell in our laboratory (unpublished results, 1965) partially explained this discrepancy by

showing that the sex difference does not occur in all strains of mice; indeed, he found no sex difference in the duration of action of hexobarbital in the NIH general purpose strain used in this study.

Our studies on the metabolism of ethyl-morphine by liver microsomes can provide an explanation for these apparently anomalous observations. In species like the rat in which the K_m is lower in the male than in the female, while the V_{max} is greater in males than in females, the sex difference would be evident at all drug concentrations. However, the present study indicates that the sex differences in drug metabolism by mouse liver microsomes would become evident at high substrate concentrations but not at the low concentrations which usually occur *in vivo*. This is easily understood when the following equation derived from the usual Michaelis-Menten equation is considered:

$$\frac{v_{(male)}}{v_{(female)}} = \frac{V_{max\ (male)}}{V_{max\ (female)}} \cdot \frac{[\ s + K_{m\ (female)}\ ]}{[\ s + K_{m\ (male)}\ ]}$$

Since the values of both the V_{max} and K_m obtained with liver microsomes of female mice were double those obtained with males, the ratio v_{male}/v_{female} approaches unity as the substrate concentration is decreased (see Fig. I).

The finding that no significant sex differences in K_m and V_{max} values occur in guinea pig, rabbit and monkey are in accordance with the lack of sex difference in the duration of hexobarbital action in these species (Quinn *et al.*, 1952). However, the results with monkeys should not be considered conclusive because their small size suggests that they are not fully grown and thus the sex difference may not have developed.

Our results also provide evidence that the activity of a drug metabolizing enzyme varies both quantitatively and qualitatively. They revealed about a four-fold variation in K_m among the various species studied and about five-fold variation in V_{max}. Thus it would be a mistake to assume that species variations in drug metabolism are due solely to differences in the amount of enzyme present in liver microsomes.

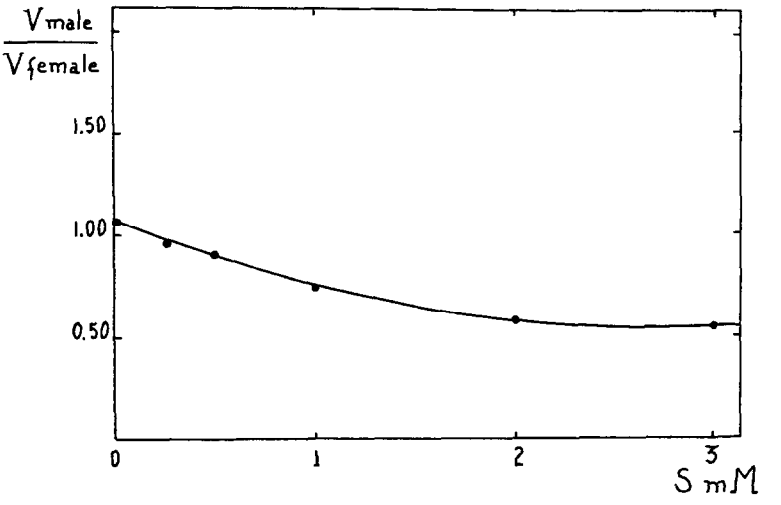

Fig. I. Effect of substrate concentration on the ratio v_{male} / v_{female} for mice. The points were obtained by giving values to s and replacing V_{max} and K_m by the data we obtained for mice.

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