

# Sex Difference in the Metabolism of Hexobarbital in the Mongolian Gerbil (*Meriones unguiculatus*)<sup>1</sup> (35998)

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A sex difference in the metabolism of many drugs by the rat liver microsomal enzyme system has been described by various reports. For example, liver microsomal enzymes of the male rat are more active in metabolism of hexobarbital than those of the female rat (1). Also, some strains of mice demonstrate a sex difference in metabolism of barbiturates as indicated by the duration of pentobarbital (2) and hexobarbital (3) hypnosis. However, in the mouse, the liver microsomal enzymes of the female appear to be more active in metabolism of the barbiturates.

Thus far, except in the two mentioned species, this phenomenon of sex differences in metabolism of drugs has not been demonstrated in any other species. In the present study, the possibility of a sex difference in metabolism of hexobarbital in the Mongolian gerbil (*Meriones unguiculatus*) was investigated.

**Methods.** Young adult male and female Mongolian gerbils of approximately 3 months of age were used. The body weight ranged from 62 to 68 g for the female and 68 to 75 g for the male.

**Hexobarbital "sleeping time."** Sodium hexobarbital (50 mg/ml) was administered intraperitoneally to the gerbils at the dose level of 150 mg/kg. The duration of hexobarbital sleeping time was defined as the time between the end of injection and the moment that the gerbils regained their righting reflex.

<sup>1</sup> A preliminary report of this work was presented Annu. Meet. Fed. Soc. Exp. Biol., Atlantic City, NJ, 1970. Part of this material appeared in a dissertation by M.D. Maines in partial fulfillment for the requirements for the degree of Doctor of Philosophy in the Department of Pharmacology, University of Missouri, Columbia.

**Preparation of microsomes and enzyme assay.** The animals were decapitated and the livers were rapidly removed, washed, and homogenized with 3 vol of ice-cold isotonic KCl solution in a Teflon-glass homogenizer. The homogenate was centrifuged at 9000g for 20 min at 2–3°. The supernatant fraction was centrifuged at 105,000g for 60 min to sediment the microsomes. The microsomal pellet was suspended in 1.15% KCl solution and used for determination of cytochrome P-450 content by the method described by Omara and Sato (4). The microsomal protein content was determined according to the method of Lowry *et al.* (5). Bovine serum albumin was used as the protein standard. The aliphatic hydroxylation of hexobarbital was measured by the method of Cooper and Brodie (6). One milliliter aliquots of 9000g supernatant fraction were incubated for 20 min under 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 38.5 ± 0.5° in a Dubnoff metabolic shaker with the following (μmoles): magnesium chloride, 25; nicotinamide, 25; glucose-6-phosphate, 60; NADP, 1.2; and hexobarbital, 4; with 3.0 ml of 0.5 M Hepes buffer (pH 7.4); the final total volume was 5.0 ml.

Statistical analyses were made using Student's *t* test. A *p* value of 0.05 or less was considered significant.

**Results. Sex difference in hexobarbital sleeping time.** Data presented in Table I show that there was a sex difference in the sleeping time of hexobarbital. The mean sleeping time of the female gerbil was 70 min in comparison with that of the male of 105 min. This difference was significant (*p* < .01).

**Sex difference in in vitro metabolism of hexobarbital.** As shown in Table II, there was a sex difference in hexobarbital hydroxylating activity of the liver 9000g supernatant

TABLE I. Sex Difference in the Duration of Hexobarbital Sleeping Time in the Gerbil.<sup>a</sup>

Sex	Mean sleeping time (min)
Male (12) <sup>b</sup>	105 ± 9.6
Female (9)	70 ± 6.9 <sup>c</sup>

<sup>a</sup> The results are given as the mean ± SEM.

<sup>b</sup> Number of animals.

<sup>c</sup>  $p < .01$ .

fraction. Hexobarbital was metabolized at a faster rate by the female than male gerbil. The content of microsomal cytochrome P-450 was significantly higher in the female. However, the ratios of liver weight to body weight and the content of microsomal protein were not significantly different in the males and the females.

*Discussion.* The existence of a sex difference in the metabolism of some drugs is an unusual phenomenon which thus far has been observed only in the rat and the mouse. In the present study it was demonstrated that there was a sex difference in the metabolism of hexobarbital in the Mongolian gerbil. The hexobarbital hydroxylating activity of the female 9000g liver supernatant fraction was found greater than that of the male which indicates a higher rate of drug-metabolizing activity by the liver microsomal enzyme system of the female gerbil. Also, the microsomal content of cytochrome P-450 was

found slightly but significantly higher in the female, which is consistent with the correlation often found between the content of microsomal cytochrome P-450 and the activity of many drug-metabolizing enzymes. However, the observed sex difference in the hexobarbital hydroxylating activity of the liver was not likely due to a sex difference in the microsomal enzyme content, since there was not a significant difference in the content of microsomal protein in the male and the female gerbils.

In general, the duration of hexobarbital sleeping time is considered indicative of the rate of *in vivo* metabolism of the drug. The present finding that the duration of hexobarbital hypnosis in the female was significantly shorter than in the male, further supports the possibility of the presence of a sex difference in the metabolism of the barbiturate in this species. Furthermore, since the ratio of liver weight to the body weight was found to be the same, the shorter duration of hexobarbital hypnosis in the female gerbil can not be attributed to the higher content of microsomal enzymes in the females due to a proportionally larger liver.

*Summary.* A sex difference was observed in the metabolism of hexobarbital in the Mongolian gerbil. The hexobarbital hydroxylating activity of the liver microsomal enzymes and the microsomal content of cytochrome P-450 were found to be higher in the female

TABLE II. Metabolism of Hexobarbital by the Liver 9000g Supernatant Fraction of the Male and Female Gerbil.<sup>a</sup>

Parameter	Male (4) <sup>b</sup>	Female (7)	<i>p</i>
Liver wt (% of body wt)	3.89 ± 0.21	3.77 ± 0.33	NS <sup>c</sup>
Microsomal protein content (mg/g)	20.74 ± 1.61	23.60 ± 2.97	NS
Microsomal cytochrome P-450 content (ΔOD (450–490 nm) × 10 <sup>3</sup> / mg of protein)	76 ± 4	87 ± 5	<.01
Hexobarbital hydroxylation (μmoles/g and 20 min)	2.01 ± 0.36	3.11 ± 0.27	<.05

<sup>a</sup> All the results are expressed as the mean ± SEM.

<sup>b</sup> Number of animals.

<sup>c</sup> Not significant.

gerbils. This was further supported by the finding that the duration of hexobarbital hypnosis was shorter in the female than in the male gerbils.

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