10. R. W. Talley and V. K. Vaitkevicious, *Blood* **21,** 352 (1963).
11. G. E. Underwood, *Proc. Soc. exp. Biol. Med.* **129,** 235 (1968).
12. H. E. Kaufman and E. D. Maloney, *Archs Ophthal., N.Y.* **69,** 626 (1963).
13. M. Y. Chu and G. A. Fischer, *Biochem. Pharmac.* **14,** 333 (1965).
14. R. I. Momparler, M. Y. Chu and G. A. Fischer, *Biochim. biophys. Acta* **161,** 481 (1968).
15. S. Silagi, *Cancer Res.* **25,** 1446 (1965).
16. W. A. Creasey, R. J. Papac, M. D. Markiw, P. Calabresi and A. D. Welch, *Biochem. Pharmac.* **15,** 1417 (1966).
17. M. Y. Chu and G. A. Fischer, *Biochem. Pharmac.* **17,** 753 (1968).
18. M. Y. Chu, *Biochem. Pharmac.* **20,** 2057 (1971).
19. R. I. Momparler, *Biochem. biophys. Res. Commun.* **34,** 465 (1969).
20. F. L. Graham and G. F. Whitmore, *Cancer Res.* **30,** 2636 (1970).
21. A. P. Kimball and M. J. Wilson, *Proc. Soc. exp. Biol. Med.* **127,** 429 (1968).
22. J. J. Furth and S. S. Cohen, *Cancer Res.* **28,** 2061 (1968).
23. G. A. Fischer and A. C. Sartorelli, *Meth. med. res.* **10,** 247 (1964).
24. M. Y. Chu, M. L. Hoovis, P. Calabresi and G. A. Fischer, *Pharmacologist* **13,** 209 (1971).
25. S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb and J. Lichtenstein, *Proc. natn. Acad. Sci. U.S.A.* **44,** 1004 (1958).
26. K. U. Hartman and C. Heidelberger, *J. biol. Chem.* **236,** 3006 (1961).
27. M. Y. Chu and G. A. Fischer, *Biochem. Pharmac.* **17,** 741 (1968).

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Effects of chronic administration of morphine on pentobarbital responses in the mouse

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The effect of morphine on hepatic microsomal activity has been reported to be species and sex dependent. In the rat, numerous studies have shown that chronic administration of morphine to rats depresses microsomal metabolism of many drugs [1 7]. Several investigators have further shown that the effect of morphine on hepatic drug metabolism could be demonstrated only in sexually mature male rats [7, 8–11]. However, the rat seems to be unique for demonstrating a sex dependency in the metabolism of drugs, since sex differences are not seen in the mouse, guinea pig, cat or dog [7, 12, 13]. Since a sex dependency in drug metabolism could not be demonstrated in the mouse, it was postulated that morphine should exert no depressant effect on the ability of male and female mice to metabolize drugs [11]. In support of the postulate, it was reported that liver microsomes obtained from mice of both sexes receiving morphine sulfate, 20 mg/kg i.p., once daily for 4 days, exhibited no significant differences on several drug-metabolizing parameters including the ability to metabolize ethylmorphine [11]. In the present communication, we present evidence that morphine can inhibit drug microsomal activity in both male and female mice, using as measurements *N*-demethylation of ethylmorphine and pentobarbital sleeping time.

Both male and female ICR mice (Simonsen Labs., Gilroy, Calif.), weighing 23–25 g, were rendered tolerant to and physically dependent on morphine by the subcutaneous implantation of a specially formulated morphine pellet [14]. Control mice were implanted with placebo pellets for the same period of time. To assess the effect of this treatment on the effect of Na-pentobarbital, both sleeping time and lethality were determined after 72 hr of pellet implantation. The sleeping time of each group was measured after a single dose of Na-pentobarbital, 60 mg/kg i.p., with twelve to fourteen mice in each group. The duration of sleeping time was taken as the time between the loss of righting reflex of the animals and the time they righted themselves. In the lethality experiments, the 24 hr LD_{50} and 95% confidence limits of Na-pentobarbital were estimated, using at least three doses of Na-pentobarbital and eight mice per dose [15]. The activity of hepatic drug-metabolizing enzymes was determined by the *N*-demethylation assay technique [16] which involves the isolation of microsomes by centrifugation and the incubation of the microsomes fortified with an NADPH-generating system in the presence of ethylmorphine. The degree of enzyme activity was indicated by the amount of formaldehyde formed from the *N*-demethylation of ethylmorphine. The number of assays per group was between six and eight.

The chronic administration of morphine by 3 days of pellet implantation potentiated the effects of pentobarbital on sleeping time and on lethality in both male and female mice. As summarized in Table 1, mice of both sexes receiving a morphine pellet implant for 72 hr exhibited a sleeping

Table 1. Enhancement of pentobarbital responses after morphine pellet implantation in the mouse

Treatment	Sleeping time (min)		LD_{50} (50% confidence limits)	
	Male	Female	Male	Female
Placebo pellet	55·8 ± 11·8	52·6 ± 5·2	120 (102·7–140·3)	115 (110·4–119·8)
Morphine pellet	141·9 ± 15·8*	236·8 ± 25·4*	86 (79·2–93·4)	86 (81·1–91·2)

* $P < 0.0005$.

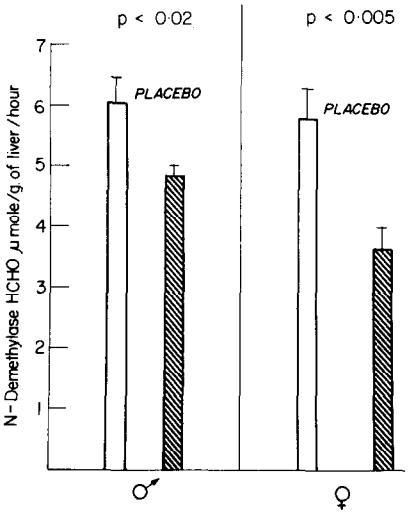

Fig. 1. Inhibition of hepatic microsomal metabolism of ethylmorphine after morphine pellet implantation in male and female mice. Mice were rendered tolerant to morphine by implantation of a 75 mg morphine pellet for 72 hr; the control groups were implanted with placeo pellets. The diagonal bars represent the group implanted with a morphine pellet. The number of assays per group was between six and eight.

time after Na-pentobarbital several-fold longer than that of the control group implanted with a placebo pellet. The toxicity of pentobarbital in the morphine pellet-implanted male or female mice was also increased, as evidenced by the decrease in LD_{50} of pentobarbital to 66 and 75 per cent of that of the control male and female animals respectively.

In confirmation of findings by others, we found that the ability of hepatic microsomal enzyme to metabolize ethylmorphine in male and female mice was the same [11]. Moreover, we have found that morphine has a significant inhibitory effect on microsomal metabolism of ethylmorphine in both sexes. As shown in Fig. 1, in a group of male mice receiving a morphine pellet for 72 hr, the hepatic microsomal metabolism of ethylmorphine was inhibited 24 per cent. A similar phenomenon was also demonstrated in female mice, the microsomal enzyme activity of morphine pellet-implanted female mice being reduced to 65 per cent of that of the placebo control group. No significant difference in hepatic microsomal metabolism of ethylmorphine could be established between the two sexes.

It can be argued that the enhancement of pentobarbital sleeping time by morphine may be attributed to an acute enhancement of an existing depression. However, it has been repeatedly determined in our laboratory that the brain levels of morphine 30 min after a single morphine dose, 10 mg/kg s.c., are comparable to those obtained 72 hr after pellet implantation. At these similar brain morphine levels, however, the enhancement of pentobarbital sleeping time by morphine after pellet implantation is about 3-fold, whereas by injection it was less than 1·7-fold (I. K. Ho *et al.*, unpublished observations). Thus, the enhancement of the prolongation in pentobarbital sleeping time by chronic morphine administration can be attributed to a large extent to inhibition of drug microsomal activity. To our knowledge, this is the first indication that chronic administration of morphine can: (1) enhance the responses to pentobarbital, and (2) decrease the metabolism of ethylmorphine in mice. The first finding might be expected and perhaps the second one also, were not predictions and findings to the contrary [11]. The fact that morphine exhibits a sex-dependent action on microsomal drug metabolism in the rat by impairing an androgen-induced stimulation of the hepatic mono-oxidase system and the fact that in the mouse a sex difference in altering drug metabolism cannot be demonstrated [11] do not have to mean necessarily that morphine cannot inhibit liver microsomal activity. Each phenomenon may occur by separate and unrelated modes in the two species. While evidence was provided on the mouse to indicate that morphine did not inhibit liver *N*-demethylase activity as measured by the ability to metabolize ethylmorphine [11], we obtained evidence to the contrary. The discrepancy can be explained by the differences in experimental conditions, ours being more drastic. The point to emphasize, however, is that there appears to be insufficient evidence at present to permit making predictions with respect to morphine effects on drug metabolism in various species.

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REFERENCES

1. J. Axelrod, *Science, N.Y.* **124,** 264 (1956).
2. G. J. Mannering and A. E. Takemori, *J. Pharmac. exp. Ther.* **127,** 187 (1959).
3. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125,** 105 (1959).
4. D. H. Clouet, *J. Pharmac. exp. Ther.* **144,** 354 (1964).
5. D. H. Clouet and R. Ratner, *J. Pharmac. exp. Ther.* **144,** 362 (1964).
6. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150,** 285 (1965).
7. R. Kato, K-I. Onoda and A. Takanaka, *Biochem. Pharmac.* **20,** 1093 (1971).
8. H. Remmer and B. Alsleben, *Klin. Wschr.* **36,** 332 (1958).
9. R. Kato, K. Onoda and M. Sasajima, *Jap. J. Pharmac.* **20,** 1941 (1970).
10. R. Kato, A. Takahashi, T. Ohshima and E. Hosoya, *J. Pharmac. exp. Ther.* **174,** 211 (1970).
11. N. E. Sladek, M. D. Chaplin and G. J. Mannering, *Drug Metab. Dispos.* **2,** 293 (1974).
12. J. S. Nicholas and D. H. Barron, *J. Pharmac. exp. Ther.* **46,** 125 (1932).
13. G. P. Quinn, J. Axelrod and B. B. Brodie, *Biochem. Pharmac.* **1,** 152 (1958).
14. E. L. Way, H. H. Loh and F. H. Shen, *J. Pharmac. exp. Ther.* **167,** 1 (1969).
15. J. T. Litchfield and F. A. Wilcoxon, *J. Pharmac. exp. Ther.* **96,** 99 (1949).
16. G. L. Mannering, in *Selected Pharmacological Testing Methods* (Ed. A. Berger), p. 51. Marcel Dekker, New York (1968).