10 ml. of AcOH. Temperature was raised to 30° and the mixture was stirred for more 7 hr. The reaction mixture was worked up as above and the crude product was recrystallized from EtOH to give X as colorless prisms, m.p. 169~170°. Yield 70%. *Anal.* Calcd. for $C_{18}H_{23}O_4N$: C, 68.12; H, 7.31. Found: C, 67.80; H, 7.49. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670. UV λ_{max}^{EtOH} mμ (log ε): 245 (4.23), 289 (4.07), 312 (3.70). $[\alpha]_D^{25.5}$ +49.6° (c=1.04, MeOH). NMR τ ($CDCl_3$): 6.00 (3H, OCH_3), 7.65 (3H, N-CH_3).

Oxidation of (−)-3-Methoxy-N-methylmorphinan (XI)——A solution of 0.9 g. of chromic acid or 1.4 g. of sodium bichromate in 10 ml. of AcOH and 6 ml. of H_2O was added during 30 minutes at 10° to a stirred solution of 1.2 g. of XI in 10 ml. of AcOH. Temperature was raised to 30°. The mixture was stirred for further 7 hr. and worked up as above to give XII as pale yellow prisms from MeOH, m.p. 192~194°. Yield 80%. *Anal.* Calcd. for $C_{18}H_{23}O_2N$: C, 75.75; H, 8.12. Found: C, 75.81; H, 8.19. UV λ_{max}^{EtOH} mμ (log ε): 232 (4.07), 287 (4.14). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670. $[\alpha]_D^{23}$ −98.6°(c=1.13, EtOH). NMR τ ($CDCl_3$): 6.22 (3H, OCH_3), 7.59 (3H, N-CH_3).

The authors are grateful to Drs. Y. Sawa, W. Nagata and K. Tori of the Shionogi Research Laboratory for their helpful guidance and discussion, and are also indebted to Drs. H. Fujimura and I. Seki of the Gifu Medical College for their kind supply of some starting materials.

Chem. Pharm. Bull. 15(9)1419~1422(1967)

UDC 615.782.54-092

Ryuichi Kato and Akira Takanaka*1: Lack of Chronic Morphine Effect on the Induction of Drug-Metabolizing Enzymes of Liver Microsomes by Phenobarbital in Female Rats.

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(Received July 23, 1966)

It has been shown that administration of phenobarbital and various drugs stimulate the activities of drug-metabolizing enzymes of liver microsomes.[1)]

On the other hand, some investigators reported that repeated administration of morphine reduced the activities of the drug-metabolizing enzymes in male rate.[2,3)] Moreover, Remmer demonstrated that chronic administration of morphine reduced the activities of the drug-metabolizing enzymes in male rats, but it stimulated the activities of the enzymes in female rats.[4)]

Furthermore, Kato and Gillette showed that single injection of morphine reduced the activities of drug-metabolizing enzymes of liver microsomes in male rats, but it did not alter the activities in female rats.[5,6)]

Okui and co-worker recently made an interesting observation that chronic administration of morphine for one month almost completely abolished the stimulatory effect of phenobarbital on the drug-metabolizing enzymes of male rats.[7,8)] Injection of morphine for 4 days did not reduce the effect of phenobarbital, but it was progressively reduced according to the duration of morphine administration.

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5) R. Kato, J. R. Gillette: Pharmacologist, **5**, 240 (1963).
6) *Idem*: J. Pharmacol., **150**, 285 (1965).
7) S. Okui, K. Minegishi: Eisei Kagaku, **11**, 143 (1965).
8) Y. Kuroiwa, K. Minegishi, S. Okui: This Bulletin, **13**, 731 (1965).

These results may suggest that the lack of phenobarbital effect in the rats treated with morphine results from a block of the mechanism of adaptation in liver microsomes to phenobarbital and this fact may be related to the development of the tolerance. Thus, it is of interest to study whether or not the chronic administration of morphine abolishes the stimulatory effect of phenobarbital in female rats similarly in male rats as reported by Okui, *et al.*

Experimental

Female rats of Sprague-Dawley strain (160~180 g.) and of Wister strain (120~130 g.) were used. Phenobarbital sodium (80 mg./kg.) was dissolved in distilled water and given intraperitoneally, while morphine was dissolved in distilled water and given subcutaneously.

Determination of Enzyme Activity——The rats were decapitated and the livers were removed and homogenized with 3 volumes of 1.15% (isotonic) KCl solution in a Teflon-glass homogenizer. The homogenate was centrifuged at $9000 \times g$ for 20 munites and the $9000 \times g$ supernatant was used as enzyme source.

A typical incubation mixture consisted of 2.5 ml. of $9000 \times g$ supernatant (equivalent to 625 mg. of liver), 20 μmole of glucose-6-phosphate, 0.4 μmole of NADP, 50 μmole of nicotinamide, 25 μmole of $MgCl_2$, 1.4 ml. of 0.2*M* phosphate buffer (pH 7.4), various substrate (aminopyrine, 5.0 μmole; hexobarbital, 3.0 μmole; N-methylbarbital, 5.0 μmole) and water to final volume of 5.0 ml. The mixtures were incubated for 30 minutes under air. The amount of N-demethylation of aminopyrine was estimated by measuring the formation of 4-aminoantipyrine according to the method of La Du, *et al.*[9]

The amount of hexobarbital metabolized was estimated by measuring the disapperance of the substrate according to the method of Cooper and Brodie.[10]

The amount of N-demethylation of methylbarbital was estimated by measuring the formation of formaldehyde according to the method of Chochin and Axelrod.[11]

Table I. Effect of Single Injection of Morphine on the Induction of Drug-metabolizing Enzyme Systems of Liver Microsomes by Phenobarbital

	Phenobarbital treatment	Aminopyrine N-Demethylation (mμmole/g./30′)	Hexobarbital Hydroxylation (mμmole/g./30′)
1) Control	−	95± 4.3(6)	883± 39(6)
2) Morphine treated	−	86± 8.1(6)	815± 58(6)
3) Control	+	358±25.8(6)	2128±125(6)
4) Morphine treated	+	301±36.4(5)	2251±208(6)

Statistical analysis :

Comparison	Aminopyrine N-Demethylation		Hexobarbital Hydroxylation	
	Diff. (%)	Signif.	Diff. (%)	Signif.
1)——2)	− 9	N.S.	− 8	N.S.
1)——3)	+277	$p < 0.01$	+140	$p < 0.01$
1)——4)	+217	$p < 0.01$	+149	$p < 0.01$
2)——4)	+250	$p < 0.01$	+170	$p < 0.01$
3)——4)	− 16	N.S.	+ 6	N.S.

Female Sprague-Dawley strain rats, weighing about 160~180 g. were used. 25 mg./kg. of morphine HCl were given 48 hr. before the sacrifice and 80 mg./kg. of phenobarbital were given 45 hr. before the sacrifice.
The results were given as averages ± standard error of the values from 6 rats.
The numerals in the brackets indicate number of the determinations.

9) B.N. La Du, L. Gaudette, N. Trousof, B.B. Brodie : J. Biol. Chem., **214**, 741 (1955).
10) J.R. Cooper, B.B. Brodie : J. Pharmacol., **114**, 409 (1955).
11) J. Chochin, J. Axelrod : *Ibid.*, **125**, 1054 (1959).

Results and Discussion

Single injection of morphine did not depress activity of the drug-metabolizing enzymes of liver microsomes in female rats as reported in previous papers.[5,6] Moreover, single injection of morphine did not prevent the stimulatory effect of phenobarbital on the drug-metabolizing enzymes of liver microsomes in female rats (Table I). These results are in accord with the results of Okui and co-worker which were observed in male rats. While, as shown in Table II, the activity of drug-metabolizing enzymes of liver microsomes likely decreases in the rats treated with morphine for one month, especially in the rats treated with constant dose of morphine.

Table II. Effect of Chronic Administration of Morphine on the Induction of Drug-metabolizing Enzyme Systems of Liver Microsomes by Phenobarbital

	Morphine treatment	Phenobarbital treatment	Aminopyrine N-Demethylation (mμmole/g./30′)	N-Methylbarbital N-Demethylation (mμmole/g./30′)	Hexobarbital Hydroxylation (mμmole/g./30′)
1)	——	−	77± 4.5(10)	108±16.3(8)	1110±146(10)
2)	Constant dose	−	55± 5.6(10)	86± 9.9(9)	693± 55(10)
3)	Increasing dose	−	59± 6.8(10)	102±10.5(10)	914± 81(10)
4)	——	+	231±26.2(9)	578±43.7(9)	2891±287(10)
5)	Constant dose	+	194±20.7(9)	424±42.3(10)	2092±279(10)
6)	Increasing dose	+	228±15.1(9)	644±40.3(9)	2975±239(10)

Statistical analysis :

Comparison	Aminopyrine N-Demethylation		N-Methylbarbital N-Demethylation		Hexobarbital Hydroxylation	
	Diff. (%)	Signif.	Diff. (%)	Signif.	Diff. (%)	Signif.
1)——2)	− 29	$p<0.05$	− 20	N.S.	− 38	$p<0.05$
1)——3)	− 23	$p<0.05$	− 6	N.S.	− 18	N.S.
4)——5)	− 16	N.S.	− 23	$p<0.05$	− 28	$p<0.05$
4)——6)	− 1	N.S.	+ 11	N.S.	+ 3	N.S.
1)——4)	+199	$p<0.01$	+435	$p<0.01$	+160	$p<0.01$
2)——5)	+257	$p<0.01$	+393	$p<0.01$	+202	$p<0.01$
3)——6)	+285	$p<0.01$	+533	$p<0.01$	+225	$p<0.01$

Female Wister strain rats, weighing about 120~130 g. were divided in 3 groups. The first group was not treated as control and the second group was treated with constant dose (30 mg./kg., *s.c.*) of morphine HCl and the third group was treated with increasing dose of morphine, the intial dose was 30 mg./kg. and increased 10 mg./kg. for every week.

After the treatments for 28 days, half rats of each group were treated with phenobarbital (80 mg./kg., *i.p.*) three hours after the morphine injection, and morphine injection was continued on 29th day and all rats were sacrified on 30th day.

All results were given as averages ± standard error of the values from 8~10 rats. The numerals in the brackets indicate number of the determinations.

The decrease in the enzyme activity in the morphinized rats may be related to the developments of tolerance and abstinence, since Akera recently reported that when morphine was given only once a day the abstinence may be occurred in the tolerance developed rats.[12] However, the stimulatory effect of phenobarbital on the drug-metabolizing enzymes in female rats was not prevented in the rats treated with

12) T. Akera : Folia Pharmacol. Jap., **61**, 84 (1965).

constant or increasing dose of morphine. For example, the administration of phenobarbital increased aminopyrine N-demethylation by 199%, 257% and 285%, in controls, morphine (constant dose) treated rats and morphine (increasing dose) treated rats, respectively.

Moreover, the activity of N-methylbarbital N-demethylation increased by 435%, 393% and 533% and activity of hexobarbital hydroxylation increased by 160%, 202% and 225% in controls, morphine (constant dose) treated rats and morphine (increasing dose) treated rats, respectively. These results are not in accord with the observation made on male rats that the chronic administration of morphine abolished the stimulatory effect of phenobarbital on the microsomal drug-metabolizing enzymes.

Kato and Gillette have shown that the drug-metabolizing enzymes of liver microsomes of male rats which are stimulated by the male sex hormone are likely unstable and they easily lose their activity under abnormal physiological conditions.[5,6,13]

Moreover, Kato and Takayanagi recently reported that the administration of morphine depressed the activities of drug-metabolizing enzymes of liver microsomes only in male rats and it did not depress the activities in female rats and female and male mice, guinea-pigs and rabbits.

From these results it may be assumed that the abolishment of the stimulatory effect of phenobarbital in morphinized male rats is likely due to an intereferance of the stimulatory action of male sex hormone and it is not likely due to direct interferance on the stimulatory action of phenobarbital.

13) R. Kato, J.R. Gillette: J. Pharmacol., **150**, 279 (1965).

[Chem. Pharm. Bull. 15(9)1422~1426(1967)]

UDC 615.721-03

Mitsuo Mizutani and Shun-ichi Naito*1: Studies on Absorption and Excretion of Drugs. XXIX.*2 Biopharmaceutical Studies on Guaiacol Glycerol Ether and Related Compounds. I. Blood Level of Guaiacol Glycerol Ether in Rabbit and Its Binding with Serum Proteins.

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(Received August 6, 1966)

Recently, the pharmacological studies of Guaiacol Glycerol Ether (GGE) and its related compounds has been studied by Yamada,[1] Fujimura,[2] *et al.*

Little work has been done on biopharmacy of GGE, while there exist considerable literatures. Morgan[3] had reported plasma level of GGE after oral administration in the dog.

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2) H. Fujimura: Presented at 17th Nippon Yakugakkai Kinki-shibu Assembly (1961).

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