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177. Toshihiko Ariyoshi*1 : Biochemical Studies on the Drug Metabolism. IV.*2 Adaptive Increases in Cyclobarbital-Metabolizing Enzyme Induced by Barbiturate Derivatives.*3

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It has been reported[1~3] that pretreatment of rats with phenobarbital and other barbiturates increased activity of the liver microsomal enzyme that metabolize hexobarbital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid) and that the long-acting barbiturates such as phenobarbital and barbital were better stimulators than the short-acting barbiturates as MHB. In general, MHB is considered to lose rapidly its activity because of its rapid metabolism in body.

In the previous study[4] on EHB-metabolizing enzyme activity, it was reported to observe the effect of the compounds which were produced by opening the barbituric acid ring of EHB. The present work was undertaken in an attempt to check the effect of introduction of methyl group into barbituric acid ring on the EHB-metabolizing enzyme.

Experimental

Materials and Methods——EHB was prepared from its Ca salt and recrystallized from H_2O. MHB and phenobarbital were obtained from Dainippon Pharmaceutical Co., Ltd. Allobarbital (5,5-diallybarbituric acid) and barbital were obtained from Toyo Pharmaceutical Co., Ltd., and Daiichi Pure Chemicals Co., Ltd. Nor-hexobarbital (Nor-MHB, 5-cyclohexenyl-5-methylbarbituric acid) was synthesized from cyclohexanone and ethyl cyanoacetate following the method of Nakamura.[5] 1-, or 1,3-substituted barbiturates were obtained by the methylation of barbiturates or 1-methylbarbiturates with dimethyl sulfate following the procedure of Butler, *et al.*[6] or Aspelund, *et al.*[7]

Kropp[8] reported 5-cyclohexenyl-5-ethyl-1,3-dimethylbarbituric acid (1,3-dimethyl-EHB) melted at 146°, but the compound obtainod by auther melted at 68~69°.*4 However, a compound of colorless plates, m.p. 146~147°,*5 was also obtained by hydrolysis of author's compound melted at 68~69° and the elementary analysis and the IR absorption spectrum of this compound were in good agreement with 1-(2-cyclohexenylbutyryl)-1,3-dimethylurea. From these facts, it seemed that the 1,3-dimethyl-EHB would be a compound melted at 68~69°.

The animals used were Wistar female rats and fed "Oriental rat diet-MN" for 1 week prior to the all experiments.

Barbiturates and 1-methylbarbiturates were injected intraperitoneally in single dose as aqueous solution containing 1.1 equiv. of NaOH, and 1,3-dimethylbarbiturates were administered intraperitoneally in single dose as a solution of propylene glycol. Control rats were injected intraperitoneally only with aqueous solution containing 1.1 equiv. of NaOH or with propylene glycol.

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*2 Part Ⅲ: This Bulletin, **12**, 1281 (1964).

*3 Presented at the 37th Meeting of Kyushu Branch, Pharmaceutical Society of Japan, in May, 1964.

*4 $C_{14}H_{20}N_2O_3$, m.p. 68~69°. *Anal.* Calcd. C, 63.62; H, 7.62; N, 10.60. Found C, 63.39; H, 7.71; N, 10.76.

*5 $C_{13}H_{22}N_2O_2$, m.p. 146~147°, *Anal.* Calcd. C, 65.51; H, 9.43; N, 11.45. Found C, 65.59; H, 9.41; N, 11.75.

1) H. Remmer : Naturwissenschaften, **45**, 189 (1958).
2) *Idem* : Arch. exptl. Pathol. Pharmakol, **235**, 279 (1959).
3) H. Remmer, B. Alsleben : Klin. Wochschr., **36**, 332 (1958).
4) Part Ⅲ : This Bulletin, **12**, 1281 (1964).
5) M. Nakamura : Yakugaku Kenkyu, **33**, 186 (1961).
6) T. C. Butler, M. T. Bush : J. Pharmacol. Exptl. Therap., **65**, 205 (1939).
7) H. Aspelund, O. Backman : Acta. Acad. Aboensis, Math. et phys., **14**, 19 (1944) (C. A., **42**, 574 c).
8) W. Kropp, L. Taub : U. S. Pat., 1,947,944 Feb. 20 (C. A., **28**, 2851²).

Animals were sacrificed 24 hours after the injection. The EHB-metabolizing enzyme activity and the duration of hypnosis by various barbiturates were determined as previously reported.[9]

Results and Discussion

As shown in Table I and II the pretreatment of female rats with Nor-MHB and EHB accelerated the metabolism of EHB and shortened the duration of hypnosis. However, the pretreatment with 1,3-dimethylbarbiturates had not such stimulatory effect. No effect was observed in the pretreatment with MHB either. On the contrary, the pretreatment with 1-methyl-EHB (1-methyl-5-cyclohexenyl-5-ethylbarbituric acid) accelerated the EHB-metabolism and the formation of 3-OH-EHB was stimulated, but its stimulatory effect was less than that of EHB.

Table I. The Effect of Various Barbiturates on the Duration of EHB-Hypnosis

Pretreatment	Dose (mg./kg.)	No. of rats	Body weight (g.)	Duration of EHB-hypnosis (min.)
Control		9	88	70±6
Nor-MHB	100	5	83	42±3[a]
MHB	100	5	84	66±7
1,3-Dimethyl-MHB	200	6	89	67±7
EHB	100	5	83	35±2[a]
1-Methyl-EHB	100	5	83	48±3[a]
1,3-Dimethyl-EHB	200	6	91	64±6

Animals received various barbiturates intraperitoneally in single dose 24 hours before the EHB (80 mg./kg.) injection. The values present mean±standard error.
a) *P* value : $P<0.05$

Table II. The Effect of Various Barbiturates on EHB-Metabolizing Enzyme Activity

Pretreatment	Dose (mg./kg.)	No. of rats	Body weight (g.)	Remained EHB (%)	Formed 3-OH-EHB (%)	Formed 3-Keto-EHB (%)
Control		8	115	62.8±0.8	12.6±0.7	1.68±0.98
Nor-MHB	100	8	118	53.6±1.1[a]	17.4±0.8	3.30±0.30
MHB	100	3	120	64.3±0.6	13.4±1.5	0.65±0.65
1,3-Dimethyl-MHB	200	5	101	62.2±1.4	17.5±0.7	0.32±0.18
Control		8	113	62.8±0.9	12.2±0.9	1.83±0.40
EHB	100	3	123	50.1±1.1[a]	24.5±0.4	5.75±0.06
1-Methyl-EHB	100	8	103	55.6±2.6[a]	20.4±1.1	3.73±0.51
1,3-Dimethyl-EHB	200	5	119	60.4±2.8	14.4±1.2	2.25±0.43

Animals received various barbiturates intraperitoneally in single dose. Twenty-four hours later, animals were sacrificed and the EHB-metabolizing activity of the liver was determined as previously reported.[9] The values present mean±standard error.
a) *P* value : $P<0.05$

From these results, it was interesting that the stimulating effect of Nor-MHB on EHB-metabolism was stronger than MHB, even though the hypnotic action of Nor-MHB was much weaker than MHB in rats in a dose used. It seemed that the stimulating action of some drugs on EHB-metabolizing enzyme activity would be independent of their pharmacological action and that such action would be affected with the difference of some chemical structures.

9) E. Takabatake, T. Ariyoshi : This Bulletin, **10**, 952 (1962).

Table III. The Effect of Various Barbiturates on EHB-Metabolizing Enzyme Activity

Pretreatment	Dose (mg./kg.)	No. of rats	Body weight (g.)	Remained EHB (%)	Formed 3-OH-EHB (%)	Formed 3-Keto-EHB (%)
Control		6	102	62.5±1.0	12.8±0.5	2.07±0.25
Barbital	100	4	110	50.5±1.5[a)]	19.0±0.5	2.63±0.26
1-Methylbarbital	100	5	109	52.4±2.0[a)]	19.5±1.4	2.71±0.25
1,3-Dimethylbarbital	200	4	103	63.5±1.4	12.5±0.5	1.38±0.24
Control		10	113	61.7±0.5	13.7±0.5	2.15±0.31
Phenobarbital	100	3	125	47.7±1.6[a)]	27.3±0.7	5.65±0.36
1-Methylphenobarbital	100	7	129	60.5±1.6	19.1±1.0	0.89±0.34
1,3-Dimethylphenobarbital	100	4	108	60.1±2.0	18.4±0.9	1.91±0.49
Control		6	96	62.8±0.6	12.6±0.7	1.62±0.31
Allobarbital	100	5	95	53.8±1.4[a)]	15.5±1.1	3.07±0.44
1-Methylallobarbital	100	4	101	59.2±1.1	14.9±1.1	3.12±0.66
1,3-Dimethylallobarbital	50	4	96	60.7±0.9	16.4±0.4	1.38±0.30

Animals received various barbiturates intraperitoneally in single dose. Twenty-four hours later, animals were sacrificed and the EHB-metabolizing activity of the liver was determined as previously reported.[9] The values present mean±standard error.
a) *P* value: $P<0.05$

It was further examined whether these phenomena were found for other barbiturates or not. As shown in Table III the pretreatment of female rats with original barbiturates, barbital, phenobarbital, and allobarbital, also markedly accelerated the metabolism of EHB and increased the formation of its metabolites. In the case of 1-methylbarbiturates except 1-methylbarbital (Metharbital), enzyme activity was less than that in the original barbiturates-treated rats. The pretreatment with 1,3-dimethylbarbiturates did not show any stimulatory effect on EHB-metabolism. However, as show in Table IV, the duration of hypnosis produced by 1-methylbarbiturates in the dose used was long as same as that by original barbiturates.

Table IV. Duration of Hypnosis of Various Barbiturates

Compound	No. of rats	Body weight (g.)	Duration (min.)
Nor-MHB	8	118	17±3
MHB	6	112	100±5
EHB	3	120	153±23
1-Methyl-EHB	8	102	102±17
Barbital	4	109	
1-Methylbarbital	5	107	188±12
Phenobarbital	3	123	152±22
1-Methylphenobarbital	7	126	250±32
1,3-Dimethylphenobarbital	4	106	25±3
Allobarbital	4	94	355±23
1-Methylallobarbital	4	100	360±34

Rats were injected intraperitoneally in single dose (100 mg./kg.). The values present mean±standard error.

From these facts, it was observed that the strength of stimulating activity induced by described barbiturates were not parallel with the length of duration of hypnosis produced by their barbiturates in the doses used. But it is wonder that stimulating activities of 1-methyl-EHB and 1-methylbarbital on EHB-metabolism differ from other 1-methylbarbiturates such as MHB.

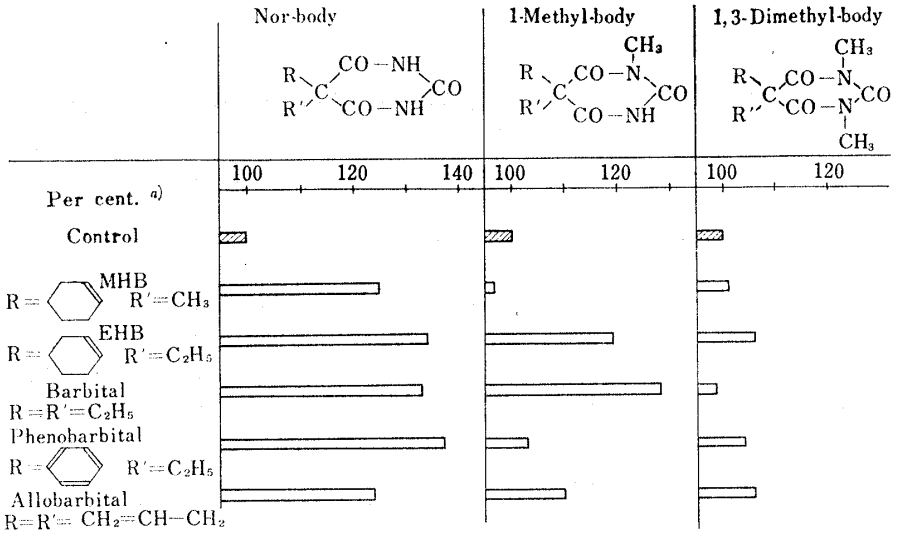

Fig. 1. Activity Ratio of Various Barbiturate Derivatives on EHB-Metabolism

a) Activity ratio given values calculated from the amount of EHB remained and expressed as per cent of the control rats.

Fig. 1 shows the relative activities of various barbiturates which were calculated from the amount of EHB metabolized in the rat liver. It was observed that the stimulatory effect of barbiturates on EHB-metabolizing enzyme activity was reduced by the introduction of methyl group into the barbituric acid ring. From these results, it was found that such inductive effect of the described barbiturates would be closely related to the substitution of N-position of barbituric acid ring.

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Summary

Various 1-methyl- and 1,3-dimethylbarbiturates were prepared and their stimulatory effect on EHB-metabolism in the rat liver preparation were examined.

The pretreatment of female rats with original barbiturates markedly accelerated the EHB-metabolism than that with 1-methylbarbiturates, and 1,3-dimethylbarbiturates treatment had not such a stimulatory effect.

It was demonstrated that such stimulatory effect of various N-substituted barbiturates on EHB-metabolism would be related to the substitution of N-position of barbituric acid ring.

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