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INCREASE OF PENTOBARBITONE METABOLISM INDUCED IN RATS PRETREATED WITH SOME CENTRALLY ACTING COMPOUNDS

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Rats treated with phenobarbitone, phenaglycodol, glutethimide, nikethamide, meprobamate, chlorbutol and chlorpromazine showed an increased metabolism of pentobarbitone and, at the same time, a diminished sleeping-time after pentobarbitone. This effect developed 24 hr after treatment, the maximum increase in metabolism occurring after about 48 hr. The increased pentobarbitone metabolism was inhibited by ethionine injected shortly before treatment. Using a liver slice preparation, increased pentobarbitone metabolism was also observed *in vitro*. These results are in accord with the view that the capacity of compounds to increase pentobarbitone metabolism may be related to their ability to act directly on microsomal enzyme systems.

In recent years it has become generally recognized that the breakdown of drugs by the liver is of great importance in determining the duration of drug action (Brodie, Gillette & LaDu, 1958). Liver damage or administration of SKF 525A (2-diethylaminoethyl diphenylpropylacetate) markedly prolongs the duration of drug action by impairment of drug metabolism (Brodie, 1956).

Recently, Remmer (1959) demonstrated that rats, pretreated with barbiturate compounds, showed a remarkable resistance to hexobarbitone concurrently with an increased breakdown of hexobarbitone by a liver microsomal preparation. Kato (1959a & b ; 1960a) reported that rats, treated 48 hr beforehand with drugs unrelated to the barbiturate series but most of which had a central action, developed a remarkable resistance to pentobarbitone. He suggested that increased breakdown of pentobarbitone by the liver microsomal enzyme was responsible.

In connexion with the development of tolerance and especially of cross-tolerance to pentobarbitone in rats pretreated with certain centrally acting drugs, it would be interesting to know whether the phenomenon is associated with an increased breakdown of pentobarbitone or with factors such as effects on the sensitivity of the central nervous system to pentobarbitone, on its distribution between serum and brain, or on its absorption or elimination. The present work is an attempt to investigate these possibilities.

METHODS

Experiments were made on female rats of the Sprague-Dawley strain weighing about 160 to 200 g. In some *in vitro* experiments, male rats weighing 60 g were used.

The rats were pretreated with one of the following drugs: phenaglycodol 130 mg/kg, thiopentone sodium 30 mg/kg, glutethimide 80 mg/kg, phenobarbitone sodium 90 mg/kg, nikethamide 200 mg/kg, meprobamate 200 mg/kg, pentobarbitone sodium 25 mg/kg, chlorbutol 110 mg/kg, chlorpromazine 15 mg/kg, fluopromazine 15 mg/kg, urethane 800 mg/kg, phenytoin sodium 100 mg/kg, paraldehyde 400 mg/kg, hexobarbitone sodium 100 mg/kg (s.c.), primidone 150 mg/kg, carisoprodol 150 mg/kg, methylpentynol carbamate 150 mg/kg, chlordiazepoxide (Librium) 50 mg/kg, promazine 20 mg/kg, hydroxyzine 150 mg/kg, imipramine 20 mg/kg, zoxazolamine 100 mg/kg, mephenesin carbamate 200 mg/kg, captodiame hydrochloride 150 mg/kg, chloral hydrate 200 mg/kg, ethyl alcohol 40% 10 ml./kg, methylpentynol 150 mg/kg, α,α-phthaloylglutarimide 500 mg/kg, bemegride 10 mg/kg, amphetamine sulphate 30 mg/kg, methyl phenidate 30 mg/kg. Ethyl ether was given by inhalation to produce general anaesthesia in 10 min.

Most drugs were dissolved in distilled water and injected intraperitoneally in a total volume of 2 ml./kg. Ethionine [α-amino-γ-(ethylthio)butyric acid] was dissolved in 0.9% sodium chloride solution. Phenaglycodol, glutethimide, meprobamate, carisoprodol, phthaloylglutarimide, mephenesin and its dicarbamate, methylpentynol carbamate and chlordiazepoxide were suspended in a 1% carboxymethylcellulose solution. Chlorbutol was dissolved in arachis oil.

Usually 48 hr after pretreatment of a group of 4 rats with the centrally acting drug ("the inducer"), various doses of pentobarbitone were injected intraperitoneally and the rats were killed 1 hr later.

The pentobarbitone concentration in serum and brain was determined by the method of Brodie, Burns, Mark, Lief, Bernstein & Papper (1953).

Liver enzyme activity was determined by measuring the amount of pentobarbitone metabolized by liver slices after an incubation period of 2 hr. The rat was decapitated and the liver immediately removed and sliced with a microtome. Sliced liver (500 mg) was suspended in a Warburg flask containing 6 ml. of Krebs phosphate buffered solution (*p*H 7.4) and 0.2 ml. of 300 μg of pentobarbitone sodium and incubated with shaking in an atmosphere of oxygen at 37° for 2 hr. At the end of incubation, the reaction mixture was homogenized with a Potter–Elvehjem-type homogenizer and 2 ml. of the homogenate was used for the determination of the pentobarbitone concentration.

Sleeping-times after pentobarbitone were taken as the duration of loss of the righting reflex. (The temperature of the experimental room was 19 to 22° C.)

RESULTS

Increased pentobarbitone metabolism after pretreatment with phenaglycodol, glutethimide or nikethamide

Increased pentobarbitone metabolism began 12 to 24 hr after injection of the inducer and was maximal for all three compounds at 48 hr. After 4 days, only with phenaglycodol was some acceleration still detected.

Increased metabolism was inferred from the sleeping-time (Fig. 1a), and from the pentobarbitone concentration in brain (Fig. 1b) and in serum (Fig. 1c). Fig. 1 shows a typical experiment in which the effect of phenaglycodol has been studied on pentobarbitone metabolism in a group of experimental and in control rats. From groups such as these the histograms of Fig. 1 were constructed.

The rate of decrease in pentobarbitone concentrations in serum and brain in rats pretreated with phenaglycodol, glutethimide or nikethamide was markedly accelerated in comparison with control rats.

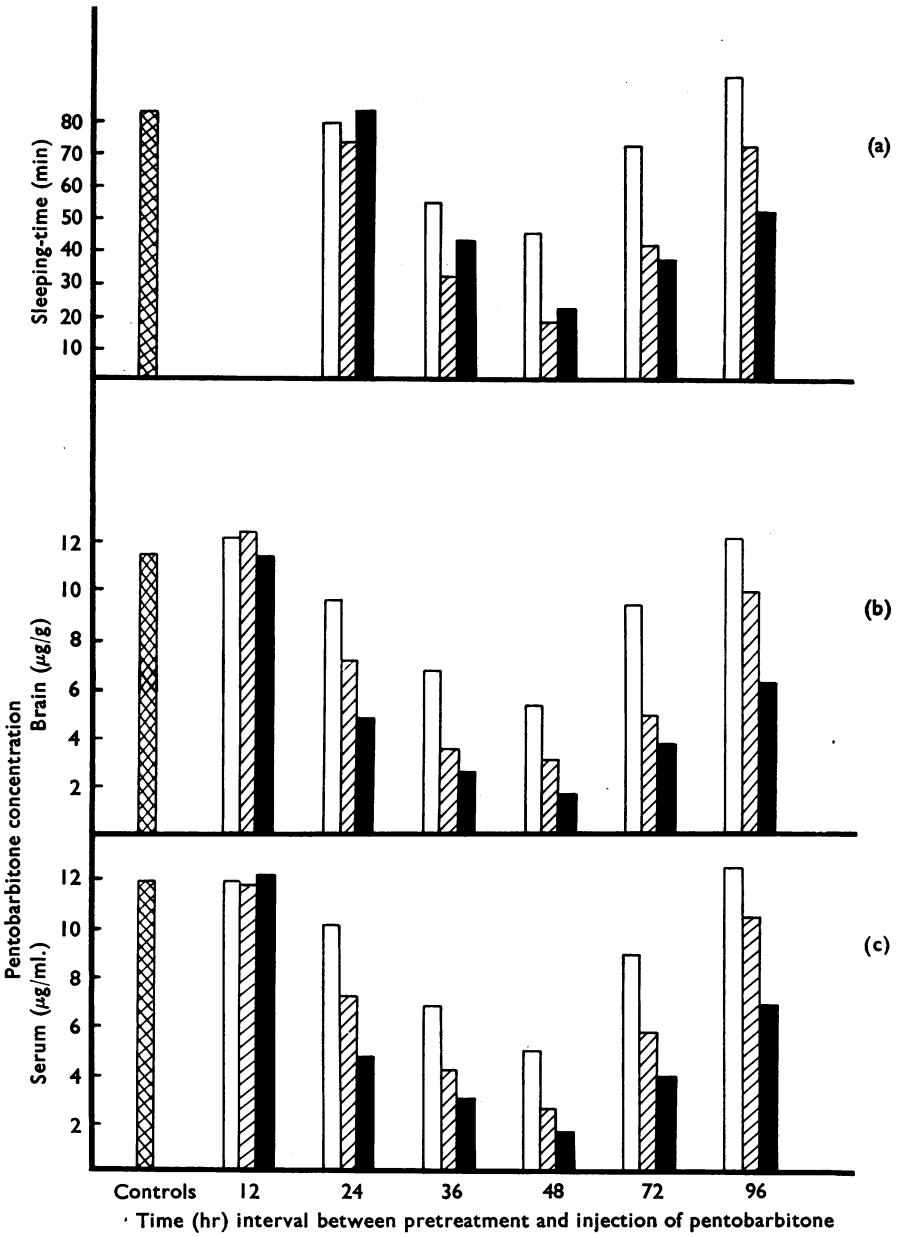

Fig. 1. The sleeping-time and brain and serum pentobarbitone concentrations of controls (cross-hatched column) and phenaglycodol, glutethimide or nikethamide-pretreated rats at different time intervals between pretreatment and injection of pentobarbitone. Rats were injected intraperitoneally with 25 mg/kg of pentobarbitone sodium after pretreatment with phenaglycodol (130 mg/kg injected intraperitoneally, black column), glutethimide (80 mg/kg injected intraperitoneally, diagonal-hatched column) or nikethamide (200 mg/kg injected intraperitoneally, open column). Determination of pentobarbitone concentration was carried out 3 hr after the injection. Pentobarbitone concentrations are given as concentrations of pentobarbitone sodium. The values are averages obtained from at least 8 animals.

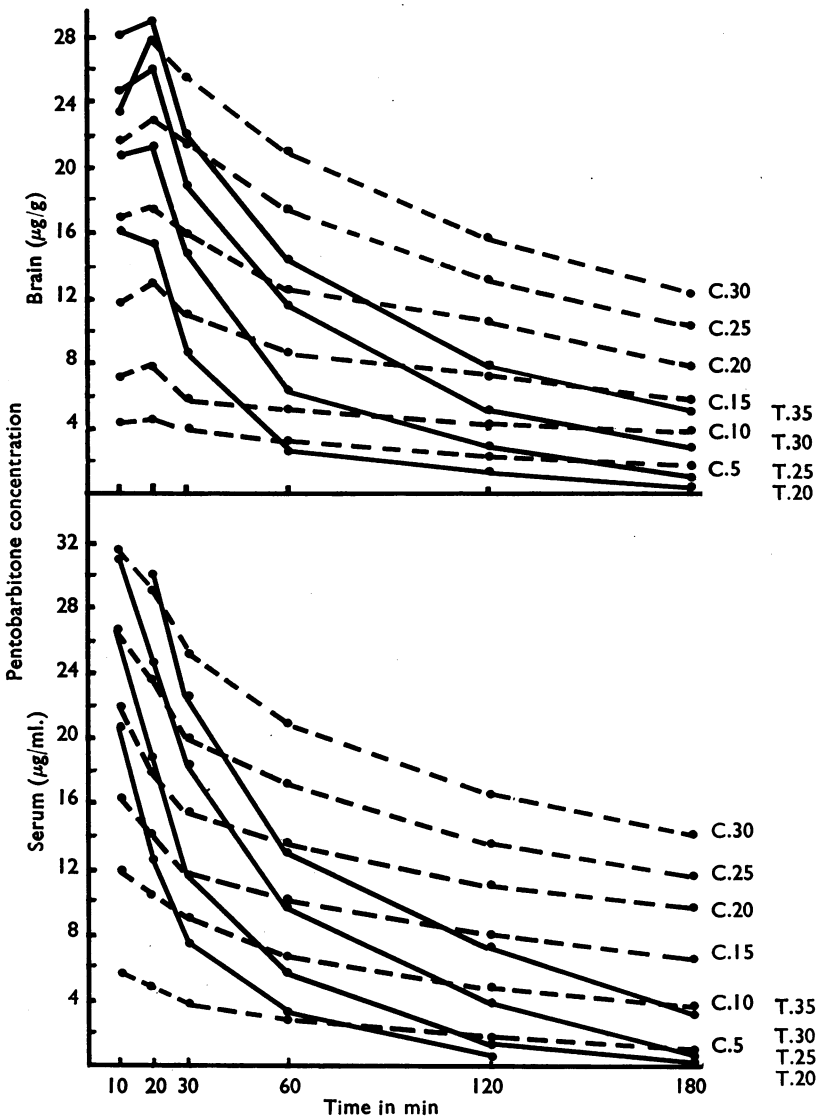

Fig. 2. Serum and brain pentobarbitone concentrations in control and phenaglycodol-pretreated rats. The rats were pretreated with phenaglycodol (130 mg/kg injected intraperitoneally) 48 hr before and after injection of various doses of pentobarbitone sodium. C.30, C.25, C.20, C.15, C.10, C.5 (●---●)=control rats injected with 30 mg/kg, 25 mg/kg, 20 mg/kg, 15 mg/kg, 10 mg/kg or 5 mg/kg of pentobarbitone. T.35, T.30, T.25, T.20 (●——●)=phenaglycodol-pretreated rats injected with 35 mg/kg, 30 mg/kg, 25 mg/kg or 20 mg/kg of pentobarbitone. Each point represents the average value obtained from at least 4 animals.

Pentobarbitone concentration in brain in relation to sleeping-time

The relation between the concentration of pentobarbitone in the brain 1 hr after injection and the duration of sleeping-time in normal, in phenaglycodol- or glutethimide-pretreated rats can be seen in Fig. 3. In normal rats (solid line),

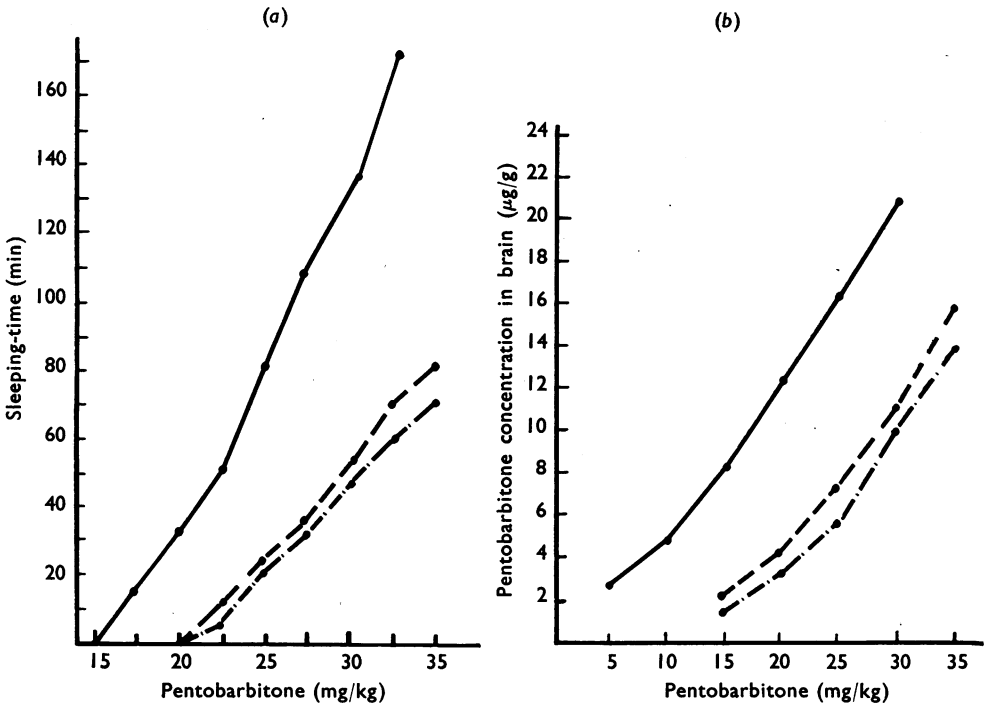

Fig. 3. Relation between the injected dose and (*a*) sleeping-times and (*b*) brain-pentobarbitone concentration in controls (● —— ●) and rats pretreated with phenaglycodol (● – · – · – ●) or glutethimide (● – – – ●). Various doses of pentobarbitone sodium were injected intraperitoneally into rats 48 hr after pretreatment with phenaglycodol (130 mg/kg injected intraperitoneally) or glutethimide (80 mg/kg injected intraperitoneally). Determination of pentobarbitone concentration was carried out 1 hr after the injection. The values given represent averages obtained from at least 4 animals for pentobarbitone concentration and at least 8 animals for sleeping-time.

23 mg/kg of pentobarbitone was required to produce sleep for 60 min (Fig. 3a). In the phenaglycodol-pretreated rats (dot-and-dash line) about 32.5 mg/kg and in glutethimide-pretreated rats (broken line) 31.0 mg/kg of pentobarbitone were necessary for the same sleeping-times.

The pentobarbitone concentrations in the brain of control rats 1 hr after receiving 20 mg/kg of the drug was 12.4 μg/g (Fig. 3b). On the other hand, it was necessary to inject 34.2 mg/kg of pentobarbitone to obtain the same concentration in phenaglycodol-pretreated rats (dot-and-dash line) and 31.6 mg/kg in glutethimide-pretreated rats (broken line).

Effect of centrally acting drugs on metabolism of pentobarbitone and on sleeping-time

Pentobarbitone concentrations in serum and brain and duration of sleeping-time in rats treated 48 hr previously with some centrally acting drugs are summarized in Table 1.

TABLE 1

EFFECT OF PRETREATMENT WITH VARIOUS CENTRALLY ACTING DRUGS AND ETHIONINE ON METABOLISM OF PENTOBARBITONE

The pretreatment with centrally acting drugs was carried out 48 hr previously and rats were given 25 mg/kg of pentobarbitone sodium by intraperitoneal injection and killed 1 hr later. Dose of ethionine is indicated in parentheses in the first column. Sleeping-time was determined after intraperitoneal injection of pentobarbitone sodium (25 mg/kg). The values are means ± standard error. The numbers in parentheses indicate the number of experiments. Serum pentobarbitone concentration, brain pentobarbitone concentration and sleeping-time of animals pretreated with drugs 2 to 17 differed significantly ($P<0.05$) from those of control animals. Pretreatment with ethionine produced no significant change ($P>0.05$) from the controls. Administration of ethionine reversed the effects of these pretreatments significantly ($P<0.05$) except when 48 hr elapsed before ethionine was administered following phenaglycodol (28)

Pretreatment I	Pretreatment II	Interval between pretreatment I and II	Pentobarbitone concentration: Serum (μg/ml.)	Variation %	Brain (μg/g)	Variation %	Sleeping-time (min)	Variation %
1. Controls			17·3±0·49 (83)		17·1±0·57 (86)		82±2·8 (153)	
2. Phenaglycodol			5·1±0·45 (16)	−70	4·7±0·34 (16)	−73	13±3·1 (24)	−84
3. Glutethimide			5·9±0·39 (15)	−66	6·0±0·25 (15)	−65	26±4·1 (20)	−68
4. Thiopentone			6·2±0·49 (12)	−64	5·8±0·42 (12)	−66	22±3·8 (15)	−73
5. Phenobarbitone			6·2±0·85 (12)	−64	4·8±0·47 (12)	−72	28±3·2 (20)	−66
6. Nikethamide			10·1±0·33 (12)	−42	9·5±0·58 (12)	−44	36±3·4 (20)	−56
7. Meprobamate			11·8±0·83 (12)	−32	10·8±0·41 (12)	−37	46±4·6 (20)	−44
8. Pentobarbitone			12·1±0·87 (8)	−30	10·5±0·41 (8)	−39	47±4·8 (12)	−43
9. Chlorbutol			12·5±0·59 (18)	−28	12·2±0·58 (8)	−29	51±6·1 (12)	−37
10. Chlorpromazine			13·0±0·88 (12)	−25	12·5±0·93 (11)	−27	49±4·5 (16)	−40
11. Fluopromazine			13·0±0·49 (8)	−25	12·5±0·75 (8)	−27	55±4·6 (13)	−33
12. Urethane			14·0±0·92 (7)	−19	14·1±0·58 (7)	−19	52±6·3 (16)	−37
13. Phenytoin			13·8±0·89 (8)	−20	13·8±0·89 (8)	−20	58±5·4 (16)	−29
14. Hexobarbitone			14·1±0·76 (8)	−19	14·0±1·05 (7)	−18	56±5·8 (16)	−31
15. Primidone			14·5±0·76 (8)	−17	13·4±1·03 (8)	−22	62±6·3 (16)	−26
16. Mephenesin dicarbamate			14·2±0·68 (8)	−18	14·2±0·58 (8)	−17	66±5·3 (16)	−19
17. Carisoprodol			14·6±0·89 (8)	−16	13·5±0·49 (8)	−22	66±4·5 (16)	−19
18. Ethionine (150 mg/kg)			15·9±1·45 (8)	−8	16·1±1·23 (8)	−8	74±5·9 (12)	−9
19. Ethionine (250 mg/kg)			17·4±1·09 (12)	+1	16·3±1·00 (12)	−7	85±6·3 (16)	+4
20. Ethionine (100 mg/kg)	Phenaglycodol	30 min	12·9±1·03 (8)	−26	13·0±0·98 (8)	−25	63±5·0 (16)	−22
21. Ethionine (150 mg/kg)	Phenaglycodol	30 min	15·4±0·98 (8)	−11	14·7±1·27 (8)	−16	65±3·9 (12)	−20
22. Ethionine (150 mg/kg)	Glutethimide	30 min	14·3±0·91 (7)	−17	14·3±1·31 (7)	−18	49±5·2 (12)	−40
23. Ethionine (150 mg/kg)	Thiopentone	30 min	16·0±1·52 (7)	−8	15·2±1·08 (7)	−13	60±4·9 (12)	−26
24. Ethionine (250 mg/kg)	Phenaglycodol	30 min	17·0±1·49 (12)	−2	16·4±0·89 (12)	−6	83±6·3 (12)	+2
25. Phenaglycodol	Ethionine	6 hr	13·1±0·73 (8)	−24	12·9±0·85 (8)	−26	60±4·9 (16)	−26
26. Phenaglycodol	Ethionine	12 hr	12·0±0·75 (8)	−31	10·9±0·56 (8)	−38	—	—
27. Phenaglycodol	Ethionine	24 hr	9·2±0·45 (8)	−47	9·0±0·44 (8)	−49	39±5·3 (11)	−48
28. Phenaglycodol	Ethionine	48 hr	6·8±0·72 (4)	−61	6.0±0·58 (5)	−65	—	—
29. Ethionine	Thiopentone	30 min	17·7±1·31 (12)	+3	17·4±1·25 (12)	−1	86±6·1 (16)	+6
30. Ethionine	Glutethimide	30 min	16·1±0·78 (12)	−7	16·0±0·93 (12)	−9	71±3·8 (16)	−12
31. Ethionine	Niketham[illegible]	30 mir	16·4±0·88 (8)	−5	16·9±1·12 (8)	−3	69±4·0 (12)	−15

The rats pretreated with phenaglycodol, glutethimide, thiopentone, phenobarbitone, nikethamide, meprobamate, pentobarbitone, chlorbutol, chlorpromazine, fluopromazine, urethane, phenytoin sodium, hexobarbitone, primidone, mephenesin carbamate and carisoprodol showed a diminished sleeping-time and a decrease in pentobarbitone concentration in brain and serum 1 hr after the injection of pentobarbitone compared with control values (Table 1). On the other hand, rats pretreated with paraldehyde, chloral hydrate, ethyl alcohol, methylpentynol, ethyl ether, α,α-phthaloylglutarimide, methylpentynol carbamate, chlordiazepoxide, promazine, hydroxyzine, captodiame, zoxazolamine, imipramine, bemegride, amphetamine and methyl phenidate showed no such change after pentobarbitone.

Effect of ethionine on the induction of increased pentobarbitone metabolism

Ethionine is a potent inhibitor of the biosynthesis of protein. It can inhibit the induction of tryptophan-peroxidase by tryptophan, the induction of the demethylase of dimethylaminoazobenzene by 20-methylcholanthrene and the induction of glucose-6-phosphatase by cortisone (Lee & Williams, 1952 ; Conney, Miller & Miller, 1956 ; Donald & Park, 1960).

When ethionine was administered 30 min before the injection of the centrally acting drug, the changes in serum and brain concentrations and sleeping-times with pentobarbitone did not occur (Table 1).

The inhibitory effect of ethionine was complete with a dose of 250 mg/kg injected intraperitoneally which produced no change in the pentobarbitone concentration in serum and brain or in sleeping-time. Partial inhibition occurred with lower doses of ethionine (100 and 150 mg/kg). The inhibitory effect of ethionine decreased as the period between the time of its injection and the administration of phenaglycodol increased. Indeed, when ethionine was administered 24 hr after the phenaglycodol, the inhibitory action of ethionine was only about 30% of its maximal value. When injected immediately before pentobarbitone, ethionine did not modify the phenaglycodol effect.

Effect of SKF 525A on metabolism of pentobarbitone in normal rats and in rats pretreated with centrally acting drugs

As SKF 525A is a potent inhibitor of the metabolism of certain drugs, including pentobarbitone (Brodie, 1956 ; Brodie, Gillette & LaDu, 1958 ; Axelrod, Reichenthal & Brodie, 1954), it was administered before injection of pentobarbitone into animals which had received no centrally acting drugs 48 hr previously. Table 2 shows that the sleeping-time and the serum and brain pentobarbitone concentrations were similar to those in the normal rats.

In vitro *metabolism of pentobarbitone by the liver of the pretreated rat*

Enzymatic activity of liver slices obtained from rats pretreated with centrally acting drugs was two or three times that of untreated rats (Table 3). Enzymatic activity was taken as the quantity of pentobarbitone metabolized by 1 g of liver slices after 2 hr of incubation. Drugs which modified neither the sleeping-time nor

TABLE 2

INHIBITION BY SKF 525A OF INCREASED METABOLISM OF PENTOBARBITONE IN PRETREATED RATS

Pretreatment was carried out 48 hr before injection of pentobarbitone (20 mg/kg injected intraperitoneally), 50 mg/kg of SKF 525A was injected intraperitoneally 30 min before injection of the pentobarbitone and the rats were killed 1 hr later. The numeral in parentheses indicates the number of animals. The values are means±standard errors

Pretreatment		Concentration of pentobarbitone					
I	II	Serum (μg/ml.)	*P*	Brain (μg/ml.)	*P*	Sleeping-time	*P*
1. Control	—	13·3±0·88 (12)		12·9±0·73 (12)		30±4·9 (16)	
2. Phenaglycodol	—	2·9±0·22 (8)	(1–2)<0·001	3·3±0·21 (8)	(1–2)<0·001	0 (16)	(1–2)<0·001
3. Thiopentone	—	4·3±0·49 (8)	(1–3)<0·001	3·9±0·30 (8)	(1–3)<0·001	2±2·3 (16)	(1–3)<0·001
4. Glutethimide	—	3·3±0·19 (8)	(1–4)<0·001	2·9±0·28 (8)	(1–4)<0·001	0 (16)	(1–4)<0·001
5. —	SKF 525A	18·9±0·52 (12)	(1–5)<0·001	18·5±0·54 (11)	(1–5)<0·001	142±9·1 (16)	(1–5)<0·001
6. Phenaglycodol	SKF 525A	17·9±0·71 (12)	(5–6) N.S.	18·0±0·61 (12)	(5–6) N.S.	98±3·7 (16)	(5–6)<0·001
7. Thiopentone	SKF 525A	19·0±0·89 (8)	(5–7) N.S.	18·3±0·75 (8)	(5–7) N.S.	102±4·3 (16)	(5–7)<0·001
8. Glutethimide	SKF 525A	18·2±0·75 (8)	(5–8) N.S.	17·5±0·49 (8)	(5–8) N.S.	89±2·9 (16)	(5–8)<0·001

TABLE 3

EFFECT OF PRETREATMENT WITH DRUGS ON *IN VITRO* METABOLISM OF PENTOBARBITONE IN RAT LIVER SLICES

Enzymatic activity is given as the quantity of pentobarbitone metabolized by 1 g of liver slices after a 2-hr incubation period. Male rats weighing 60 g were used as controls. The ethionine was injected 30 min before the injection of the inducers

Pretreatment	Dose mg/kg	No. of animals	Enzymatic activity	Variation %	*P*
Control		14	186±4·2		
Phenaglycodol	100	6	373±3·8	+100	<0·001
Thiopentone	27	4	329±7·3	+76	<0·001
Phenobarbitone	70	6	462±5·9	+138	<0·001
Pentobarbitone	23	4	291±8·1	+56	<0·001
Glutethimide	60	6	342±6·2	+84	<0·001
Nikethamide	160	4	267±4·1	+44	<0·001
Primidone	160	4	305±9·5	+59	<0·001
Meprobamate	160	4	281±4·7	+51	<0·001
Ethionine	250	4	168±6·9	−10	N.S.
Ethionine+ phenaglycodol		4	193±13·4	+4	N.S.
Ethionine+ glutethimide		4	180±7·0	−5	N.S.

the pentobarbitone concentrations did not alter the *in vitro* enzymatic activity of liver slices. When ethionine was administered 30 min before the injection of the centrally acting drugs, increased enzymatic activity was no longer observed.

As hormonal regulation of some liver enzyme activity has been recognized recently, such regulation might modify the effects of centrally acting drugs on pentobarbitone metabolism. However, hypophysectomized rats, adrenalectomized rats and gonadectomized rats behaved similarly to unoperated control rats as far as pentobarbitone metabolism was concerned.

Adrenaline (0.5 mg/kg), noradrenaline (0.5 mg/kg), acetylcholine (10 mg/kg) and aspirin (500 mg/kg) all injected intraperitoneally and histamine (7 mg/kg) injected subcutaneously, and electrical stimulation (110 V, duration of 1 sec) did not modify the pentobarbitone metabolism 48 hr later.

DISCUSSION

The results suggest that many centrally acting drugs may modify the effect of pentobarbitone in the rat. Phenaglycodol and glutethimide did not modify the metabolism of pentobarbitone *in vivo* until 12 hr after the injection (Fig. 1), nor did these drugs modify the metabolism of pentobarbitone by liver slices when added to the incubation medium.

There seems to be no alteration in the sensitivity of the central nervous system to pentobarbitone in rats pretreated with the centrally acting drugs. The diminished effect of pentobarbitone could be accounted for by an increased breakdown of pentobarbitone by liver enzyme.

Recently, Remmer (1959) reported an increased metabolism of hexobarbitone in a liver microsomal preparation of rats pretreated with barbiturate compounds. The results reported here are in accord with those of Remmer (1959). Our studies suggest that this effect may not be limited to this class alone of centrally acting drugs.

The drugs active in this study have been found (Kato, 1959a, 1960b & 1961a) to modify the pharmacological effects and to induce an increased metabolism of, for example, hexobarbitone, meprobamate, carisoprodol, strychnine, picrotoxin, schradan (octamethylpyrophosphoramide; OMPA), mephenesin, tubocurarine, phenytoin and chlorpromazine. On the other hand, Conney, Davidson, Gastel & Burns (1960) reported similar effects with hexobarbitone, zoxazolamine and amidopyrine after pretreatment with phenobarbitone and some carcinogenic substances. Our observation offers indirect support to these findings.

The mechanism of the effects reported here is not yet clear. The capacity to produce these effects is related neither to the chemical structure nor to the pharmacological effect of the centrally acting drug. It is probable that the mechanism is related to metabolism by the liver, for most of the active drugs are metabolized by microsomal liver enzymes.

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