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THE INFLUENCE OF THYROID STATUS ON THE EFFECTS AND METABOLISM OF PENTOBARBITAL AND THIOPENTAL

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Abstract—In mice, thyroid feeding increases the response to intraperitoneally injected pentobarbital and thiopental, as demonstrated by increased mortality rates, prolonged sleep, and decreased activity. Pentobarbital is removed more slowly from the brain, liver, and plasma of thyroid-fed mice than from control mice. Thyroid-fed mice sleep longer from a given dose of pentobarbital. When they waken their tissue levels are the same as those found in control mice upon wakening. Thus, delayed drug removal (rather than heightened nervous system sensitivity) appears to explain increased barbiturate response in thyroid-fed mice.

In mice propylthiouracil (PTU) feeding decreases the response (shorter sleep, less decrement of activity) to pentobarbital but not to thiopental. PTU feeding hastens the removal of pentobarbital from tissues. PTU-fed mice waken earlier than controls, at higher tissue levels. It is postulated that PTU induces increased activity of pentobarbital-destroying but not of thiopental-destroying enzymes. PTU may also have a direct action (decreased sensitivity to pentobarbital) on the nervous system.

In rats thyroxin injection only slightly increases sleeping time and only slightly delays removal of pentobarbital from tissues after intravenous injection. Thyroidectomy grossly prolongs sleeping time and grossly delays removal of pentobarbital from tissues. Thus, in the rat, hyperthyroidism and hypothyroidism have similar qualitative effects on pentobarbital response. Thyroxin-injected, thyroidectomized, and control rats sleep for different periods of time after pentobarbital injection but waken at nearly identical tissue levels. Thyroid status appears to have no effect on the sensitivity of the nervous system of the rat to pentobarbital.

A VARIETY of factors affects the intensity or duration of barbiturate action in laboratory animals. Barbiturates metabolized by side-chain oxidation by liver microsomes have an increased sedative effect after pretreatment with uracil;[1] chlortetracycline; 2, 4-dinitrophenol; 2, 4-dichlorophenoxyacetic acid;[2] and thyroid feeding.[3, 4] The same barbiturates show decreased sedative action after pretreatment with diuretics and dehydration[5] and propylthiouracil (PTU) feeding.[4] Ellinwood and Prange[6] found that both epinephrine and thyroid feeding increased sleeping time after pentobarbital, but there was no additive effect when both pretreatments were used. Again, PTU-fed mice slept a shorter time than controls. Like controls, their sleeping time could be increased by epinephrine pretreatment.

Since the present paper is concerned with thyroid status, it is of interest to note its effects on the response to drugs other than barbiturates. Carrier and Buday[7] compiled a list of diverse substances whose actions are increased by hyperthyroidism.

In addition Prange *et al.* showed an increased response to imipramine;[8] atropine, pyribenzamine, and neostigmine;[9] and desmethylimipramine.[10] PTU feeding had an opposite effect on the response to the same drugs. In this context, the report of Timeras and Woodbury[11] that PTU has a direct depressant activity on the central nervous system of the rat is pertinent.

Thyroid status is only one of many variables that affect barbiturate action; and thyroid status influences the action of many substances other than barbiturates. In the present paper we are concerned with barbiturate–thyroid relationships. Table 4, which summarizes the entire experimental results, will acquaint the reader with the scope of this work

METHODS

Mice

Male Swiss-Webster mice weighing about 25 g were housed ten per cage. Mice in certain cages were given 0·2% PTU in their drinking water for 30 days before experimentation, to produce hypothyroidism. (Other possible PTU effects will be considered.) Mice in certain cages received unadulterated food and water and were considered euthyroid controls. Mice in other cages were fed 2% desiccated thyroid in their food to produce hyperthyroidism.

Barbiturates for injection were freshly prepared in 0·9% saline from stock solution (sodium pentobarbital) or from crystals (sodium thiopental in sodium carbonate). In a given experiment all mice were injected with the same solution and usually with the same syringe. Mice from the various cages were injected in random order to avoid possible time-of-day influence on the results. All mice were injected intraperitoneally, and no mouse was used more than once.

Dose-mortality experiments were performed by injecting groups of animals with various doses of drug. In the *sleep experiments* a mouse was injected (pentobarbital, 50 mg/kg; or thiopental 55 mg/kg) and at once placed alone in a metal cage. Once asleep, the mouse was placed in the center of a circle, 9 inches in diameter, inscribed on white paper placed on a well-lighted surface, in a room at constant temperature. It was judged awake when it had removed all four feet from the circle. Sleep was measured to the nearest quarter minute as time elapsed since time of injection. This end point proved to correlate well with recovery of the righting reflex, but it was more easily discerned by the experiments while watching several mice simultaneously.

In the *activity experiments* five cage mates were injected at 15-sec intervals and placed at once in a closed, dark, doughnut-shaped runway. Six infrared beams traversed the runway, and, each time a beam was broken by the movement of a mouse, the impulse was recorded on a counter. Thus total activity per minute was recorded for each group. Activity was also recorded per 10-sec intervals for all groups, to explore the possibility of between-group differences in drug absorption rate.

In the *tissue-level experiments*, mice were injected i.p. with pentobarbital, 50 mg/kg, according to a predetermined unbiased schedule. After injection, at intervals varying from 1 to 120 min, they were killed by guillotine and exsanguinated into tubes containing oxalate. Three or four mice per time period contributed blood about

equally to serum tubes. Livers and brains were removed at once, weighed, frozen, and stored for pentobarbital determination within two weeks. Plasma was separated by centrifugation and assayed the same day.

In *another tissue level experiment*, mice were injected with pentobarbital and left alive until they wakened. They were then killed as described above. The three (or four) hyperthroid mice, for example, that wakened consecutively contributed to a given plasma pool. Their mean sleep time was correlated with a pentobarbital level representing approximately their mean plasma value. During each experiment, one group of mice from each thyroid treatment group was exsanguinated and their pooled serum assayed for radioactive triiodothronine (^{3}T) uptake. (This was done in the radiology laboratory under the direction of Dr. Francis D. Pepper, to whom the authors are indebted.) The mean results for each treatment group are as follows : hyperthyroid, 74·1%; controls, 44·5%; hypothyroid, 41·1%. It can be seen that, according to this measure, the thyroid-fed mice were grossly hyperthyroid, while the PTU-fed mice were only minimally hypothyroid.

Rats

Difficulty in performing thyroidectomies on mice and the need to pool certain mouse tissues were limitations that prompted us to use rats in continuing our work. Male Sprague-Dawley rats weighing about 75 g were selected. They were thyroidectomized under light pentobarbital–atropine anesthesia and subsequently fed Remington low-iodine diet, with 1% calcium lactate in their drinking water. Other rats were anesthetized, sham operated, and later fed standard lab chow and tap water. On the four days preceeding the experiment, rats to be made hyperthyroid were injected i.m. with 300 μg Na-l-thyroxin freshly prepared in 0·01 N NaOH. All other rats were injected with 0·01 N NaOH alone. All rats were weighed weekly, housed separately under identical environmental conditions, and given identical handling.

In the *tissue-level experiment*, all rats were injected i.v. with pentobarbital (30 mg/kg) according to a predetermined unbiased schedule. After injection, at intervals varying from 5 min to 4 hr, the rats were killed by neck fracture and guillotine, and exsanguinated into individual tubes containing oxalate. Plasma, brain, and liver were handled as described above.

In *another tissue-level experiment*, control, thyroidectomized, and thyroxin-injected (300 μg) rats were injected with pentobarbital i.v. and allowed to sleep undisturbed until they awoke spontaneously (crawled from circle). They were then killed at once. Their sleeping times were recorded individually and their plasma, brains, and livers treated as before.

In a *final tissue-level experiment*, rats were injected with 100, 300, 600 or 1,000 μg Na-l-thyroxin i.m. daily for 4 days. They were then injected with pentobarbital i.v. and allowed to sleep until they awoke. They were then killed and their tissues assayed. In addition, ^{3}T uptake was measured in their serum, and their washed hearts were weighed and compared to total body weight.

Uptake of ^{3}T by serum of a number of rats from each thyroid pretreatment group was determined. The mean values were as follows : hyperthyroid (300μg), 75·0%; control, 68·1%; hypothyroid, 23·1%. According to this measure, thyroxin-injected rats were minimally hyperthyroid, whereas thyroidectomized rats were grossly

hypothyroid. These data with the comparable data pertaining to mice, must be borne in mind when comparing experimental results between species.

Barbiturate concentration was determined in the following manner. The tissue samples of 2–3 g were homogenized in glass–Teflon homogenizers with 4 volumes of 0·1 M borate buffer, pH 9·4. Five ml of the homogenate was extracted with 22 ml of water-saturated ethyl ether, and 10 ml of the ether phase was transferred to a fresh tube containing 5 ml of 0·5 N NaOH previously saturated with ether. After shaking, the absorbency of the aqueous phase was determined with an ultraviolet spectrophotometer at 255 and 275 mμ. The reading at 275 mμ was subtracted from the reading at 255 mμ. Standard solutions were used with the method to quantify the readings. Ninety-five per cent recovery was demonstrated by addition of standards to tissues. In every experiment, tissues from uninjected animals were analyzed to obtain blank values, which were subtracted from those of the barbiturate-treated animals. Blank values were usually equivalent to readings obtained from 3–4 μg pentobarbital/ml.

The above procedure is specific for unchanged barbiturate. Pentobarbital is metabolized by hydroxylation of the butyl side chain.[12] Metabolites are much less soluble than the parent compound on organic solvents. At pH 9·4 no metabolites are extracted into ether.[13]

RESULTS

Mice

Mortality rates and sleep times. Table 1 summarizes these data. Mortality rates were statistically analyzed by the method of Litchfield and Wilcoxson.[14] Thyroid feeding decreased the LD_{50} of both pentobarbital and thiopental. The effect of PTU feeding on the LD_{50} was slight and statistically insignificant.

TABLE 1. THE EFFECTS OF ALTERED THYROID STATUS ON MORTALITY AND SLEEPING TIME

	Thy*	C†	PTU†	Thy	C	PTU	Thy vs. C	PTU vs. C
	(no. of mice)			(Estimated LD_{50})			Significance of difference between groups (P)	
Pentobarbital	62	79	75	99	110	115	<0·05	n.s.
Thiopental	77	69	81	110	140	130	<0·05	n.s.
				Mean sleeping time in min ± S.E.				
Pentobarbital	20	20	20	35·0 ±7·8	24·6 ±5·9	16·6 ±4·7	<0·01	<0·05
Thiopental	20	20	20	129·4 ±24·4	34·3 ±7·7	31·9 ±8·5	<0·001	n.s.

* Thy = thyroid fed.
† C = controls.
† PTU= propylthiouracil fed.

Thyroid feeding prolonged sleep after injection of pentobarbital and after injection of thiopental. PTU feeding shortened sleeping time after pentobarbital. It had no reliable effect on sleep time after thiopental.

Activity. Figure 1 compares the activity of the three experimental groups after injection of pentobarbital. The drug profoundly decreased the activity of thyroid-fed mice. All were heavily sedated or asleep. The PTU-fed mice were least affected.

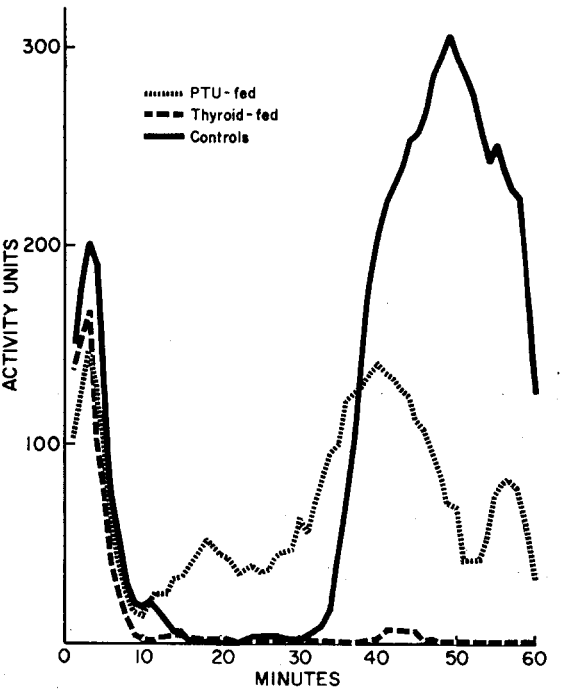

FIG. 1. Activity of mice after i.p. injection of pentobarbital (35 mg/kg). Each curve represents the combined activity of five mice.

Control mice showed an intermediate response. Their burst of activity beginning 30 min after injection almost certainly represents excitement following recovery from deep barbiturate sedation. The same phenomenon probably would have been shown by the PTU-fed mice had they been as profoundly sedated; the effect was shown by the thyroid-fed mice when they finally awakened after the termination of the experiment.

Figure 2 presents similar data pertaining to thiopental injection. Again the thyroid-fed mice were profoundly affected. With this drug, however, there was no clear difference in activity inhibition between PTU-fed mice and controls. This result is consistent with the failure of PTU clearly to alter mortality rate or sleeping time after thiopental.

The data presented in Figs. 1 and 2 were not statistically analyzed, because differences between groups, when present, were gross. However, both experiments were repeated twice with similar results. For clarity of presentation, the raw score

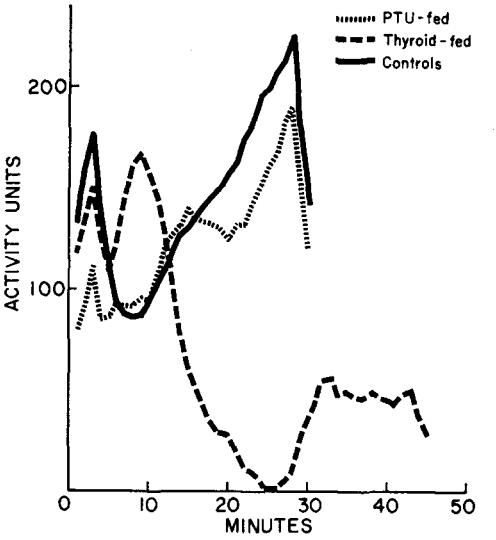

FIG. 2. Activity of mice after i.p. injection of thiopental (45 mg/kg). Each curve represents the combined activity of five mice.

for each minute was averaged with the two scores preceding it and the two following it. By this device, all curves were smoothed.

Tissues. Since thyroid feeding and PTU feeding had shown opposite effects on the response to pentobarbital (mortality rate, sleep time, activity), this drug was chosen in preference to thiopental for tissue studies.

Figure 3 shows the rate of disappearance of pentobarbital from the brains of mice in the three experimental groups. It can be seen that the drug disappears from the brain of PTU-fed mice fastest, from thyroid-fed mice slowest, and from control mice at an intermediate rate. Though not shown in the figure, at any given point in time, liver concentration of drug was higher and plasma concentration was slightly lower. The slopes for brain, liver, and plasma were approximately parallel within each pretreatment group.

Another experiment was performed to extend these findings. Mice were prepared, injected, and their sleeping times recorded as before. Upon wakening, they were sacrificed and their tissue concentrations determined (Table 2). Differences in sleeping time (Table 1) were confirmed. Thyroid-fed and control mice wakened at nearly identical tissue levels, though the time required to obtain these levels in thyroid-fed mice was greatly prolonged. PTU-fed mice wakened earlier (as expected) but at higher tissue levels. This suggested that PTU exerts some other effect in addition to hastening the removal of pentobarbital from tissues.

Rats

Since PTU had rendered mice only mildly hypothyroid and since it appeared to exert a variety of effects, it seemed advisable to pursue this work in rats, which can easily be thyroidectomized. Ease of intravenous injection in the rat offered the additional advantage of excluding differences in drug absorption as an uncontrolled variable.

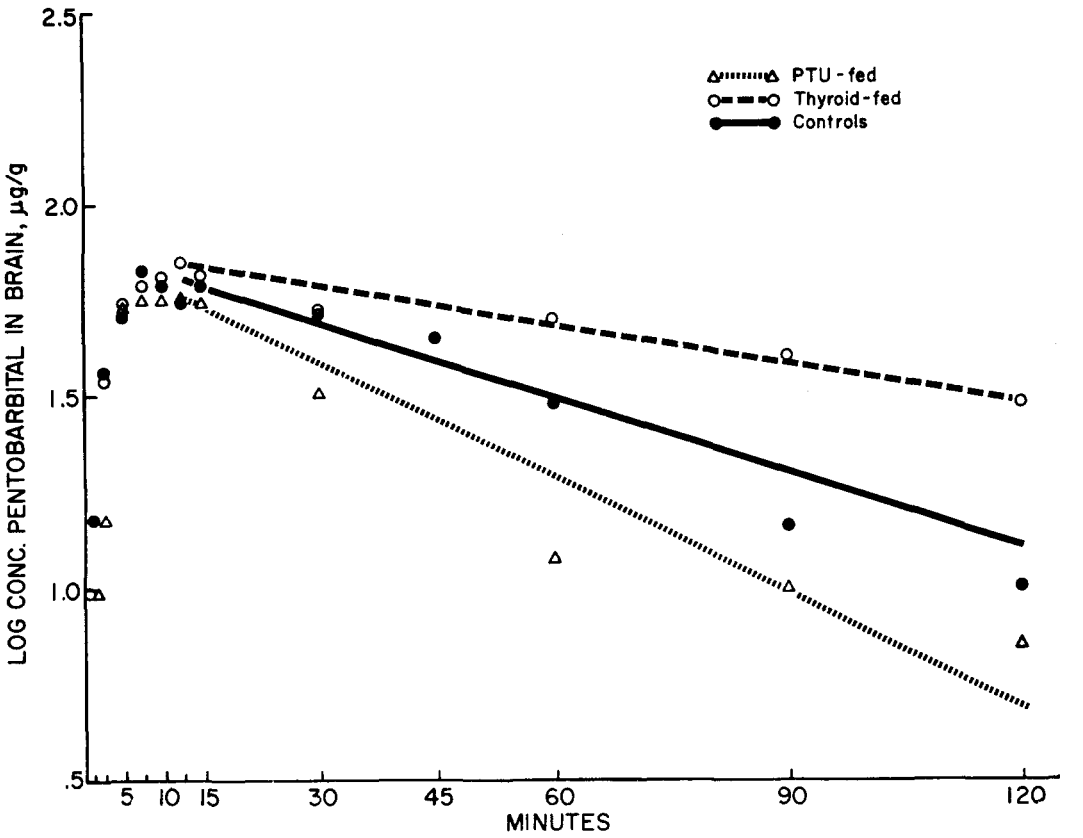

FIG. 3. The concentration of pentobarbital in the brains of mice after i.p. injection (50 mg/kg). Each point represents the mean values from three mice.

Tissues. Figures 3 and 4 (mouse brain and rat brain) can be compared. Pentobarbital disappeared more slowly from the brain of thyroidectomized rats than from the brains of other rats. The slopes for brain, liver, and plasma were approximately parallel for each pretreatment group. Thus thyroidectomy (in rats) has an effect on pentobarbital metabolism opposite to that of PTU (in mice). Thyroid injection (300 µg daily) had no effect on rate of removal of pentobarbital from tissues.

Rats were prepared, injected, and their sleeping times recorded as before. Table 2 shows that thyroidectomy remarkably prolonged sleeping time (in contrast to PTU shortening of sleeping time in mice). Thyroxin injection prolonged sleeping time slightly. All rats wakened at nearly identical tissue levels, though they required varying lengths of time to attain these levels. Thus, there is no evidence for differences in sensitivity to pentobarbital between groups to account for observed differences in response. Differences in metabolism of the drug seem an adequate explanation.

The relative failure of thyroxin injection in rats (versus thyroid feeding in mice) to delay pentobarbital metabolism was further investigated. Five groups of rats were given saline injection or various doses of daily thyroxin injection (100, 300, 600, 1,000 µg) for four days. They were then injected with pentobarbital, their sleeping times measured, sacrificed, and tissue concentrations of drug determined. Uptake by

TABLE 2. SLEEPING TIME OF MICE AND RATS AFTER PENTOBARBITAL INJECTION AND TISSUE CONCENTRATION OF DRUG UPON AWAKENING

	Mice (50 mg/kg)			Rats (30 mg/kg)		
	PTU	C	Thy	C	Thy	T'ect.
Sleeping time*	24·5 ±2·9	70·8 ±8·3	149·1 ±20·6	70·0 ±3·0	77·5 ±7·5	238·3 ±27·7
Liver†	70·0 ±4·2	52·1 ±1·4	48·0 ±2·1	66·2 ±3·4	67·0 ±2·8	67·2 ±2·4
Brain†	39·5 ±7·8	24·0 ±7·1	22·5 ±2·1	29·3 ±1·3	28·0 ±2·2	27·3 ±2·0
Plasma†	35·5 ±2·1	24·1 ±4·2	24·5 ±3·5	14·5 ±0·8	13·3 ±1·1	14·2 ±0·7

* Sleeping time in minutes ± S.E.; T'ect. = thyroidectomized.
† Pentobarbital, μg per g or ml tissue.

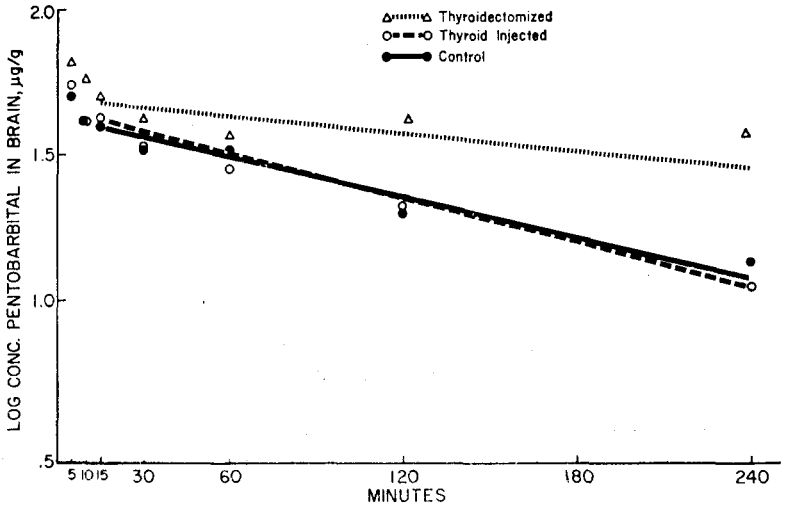

FIG. 4. The concentration of pentobarbital in the brains of rats after i.v. injection (30 mg/kg). Each solid point represents the mean value from six control rats. Other points represent means from four rats.

serum of ^{3}T, and heart weight as percent of body weight, were measured to determine hyperthyroidism. Increasing doses of thyroxin produced increasing degrees of hyperthyroidism. It also produced a slight, stepwise prolongation of sleeping time. However, tissue levels of pentobarbital for all groups upon wakening were nearly identical. The results of this experiment are shown in Table 3.

DISCUSSION

Interactions of thyroid status and thiopental and pentobarbital are summarized in Table 4. Some of the differences reported, e.g. the effect of thyroid feeding on pentobarbital mortality rate ($P < 0·05$), are slight but are supported by consistent findings on other measures of drug effect, e.g. sleep and activity.

TABLE 3. VARIOUS DEPENDENT VARIABLES AS A FUNCTION OF INCREASING THYROXIN ADMINISTRATION

Thyroxin* (μg)	Pentobarbital† (μg)			Heart wt. as % body wt.	Sleep time (min)	^{3}T uptake of serum (%)
	Plasma	Brain	Liver			
Saline	11·0 ± 0·8	27·8 ± 1·2	58·8 ± 7 2	0·37 ± 0·03	67·8 ± 1·4	69·1
100	11·2 ± 0·6	28·0 ± 0·7	59·1 ± 5·0	0·39 ± 0·02	68·0 ± 12·9	69·5
300	11·2 ± 0·4	26·5 ± 1·3	59·0 ± 2·9	0·46 ± 0·02	68·0 ± 13·5	75·0
600	13·1 ± 0·6	30·2 ± 1·0	65·0 ± 4·2	0·47 ± 0·07	76·6 ± 15·9	77·9
1,000	11·6 ± 0·5	30·2 ± 1·0	54·0 ± 1·6	0·60 ± 0·09	77·0 ± 13·1	78·3

* Thyroxin was injected i.m. for four days. Three rats in each group.

† Per g or ml of tissue. Rats were injected i.v. (30 mg/kg) and killed when they wakened.
All values are expressed as means with S.E.

TABLE 4. SUMMARY OF RESULTS; DURATION OR DEGREE OF BARBITURATE EFFECT

		Mice		Rats	
		Thy* > C	PTU† < C	Thy > C	T'ect.† > C
	Behavior				
Pentobarbital	Mortality	yes	no		
	Sleep	yes	yes	slight	yes
	Activity	yes	yes		
Thiopental	Mortality	yes	no		
	Sleep	yes	no		
	Activity	yes	no		
	Tissues				
Pentobarbital	Liver	yes	yes	slight	yes
	Brain	yes	yes	slight	yes
	Plasma	yes	yes	slight	yes

* Thy = thyroid fed (mice) or injected (rats).
† PTU = propylthiouracil fed.
† T'ect. = thyroidectomized.

Both thiopental and pentobarbital show increased effects in the hyperthyroid mouse; and pentobarbital also shows, to a lesser degree, increased effect in the hyperthyroid rat. In both species this seems to be a matter of decreased rate of drug metabolism. The increased liver:body-weight ratio of hyperthyroid animals by itself would be expected to show an opposite effect from that observed. If thyroxin and barbiturates can both be assumed to uncouple oxidative phosphorylation, hyperthyroid animals might be expected to be more sensitive to given tissue concentrations of barbiturate. However, there is no present evidence to support this point of view; i.e. there is no evidence for differences in sensitivity to given tissue levels.

Increased rate of absorption by hyperthyroid mice, though possible, could not account for prolongation of drug effect. In any case we could find no evidence for differences in rate of absorption of intraperitoneal doses of barbiturates by measuring inhibition of activity at 10-sec intervals. Absorption factors were excluded from the rat experiments by using intravenous injection.

Thyroid-induced changes in fat content may contribute to the findings, but this is made less likely by the observation that the mortality, sleep, and activity responses to thiopental and pentobarbital are increased about equally by hyperthyroidism, whereas their localization in fat is quite different.[15]

Differences in the rate of removal of pentobarbital from the tissues seems to be an adequate explanation of the phenomena observed. This is most likely the result of thyroxin decreasing the activity of the pentobarbital- (and probably thiopental-) metabolizing enzyme system. Conney and Garren[16] have shown such an effect of thyroxin on the hexobarbital-metabolizing system in the rat.

In mice, thyroid feeding greatly prolonged sleeping time whereas in rats even large doses of thyroxin had only a slight effect. This may represent a species difference in enzyme susceptibility to thyroxin, though a less profound degree of hyperthyroidism produced in the rats is an adequate explanation. It would appear that many enzyme systems[17] are more sensitive (increase or decrease) to the effects of thyroxin than is the

pentobarbital-metabolizing enzyme. It has recently been shown that glucose-6-phosphate dehydrogenase is exquisitely sensitive (increase) to thyroxin in rat liver and kidney.[18]

PTU-fed mice are less responsive than controls to pentobarbital. Thyroidectomized rats, on the other hand, show an enhanced response to pentobarbital. From this it is clear that the observed effects of PTU cannot be due to hypothyroidism per se. Ramwell and Lester[5] have shown that the acute administration of various diuretics (including sodium chloride, 200 mg/kg) will shorten the sleeping time of mice given hexobarbital. The 0·2% PTU solution used in this experiment contained 28 mEq sodium/liter. The animals in a few cages did show signs of diuresis on the first day of administration; most did not. During the remaining 30 days of treatment, fluid consumption was about equal for all groups. In any case, it would appear unlikely that any diuretic effect would manifest itself in relation to response to pentobarbital and not to thiopental (Table 1, Figs. 1 and 2).

A possible explanation of the PTU effect described is that the drug induces increased activity of the pentobarbital-metabolizing enzymes. If this is true, the failure of PTU to increase the activity of thiopental-metabolizing enzymes requires explanation. Such a degree of specificity of an enzyme-inducing substance appears possible, since Conney and Burns[19] showed that in rat liver, 3,4-benzpyrene selectively stimulates the oxidative N-demethylation of aminoazo dyes without stimulating the N-demethylation of aminopyrine or diphenhydramine.

The data show that PTU-fed mice not only metabolize pentobarbital faster than controls (Fig. 3) but also waken at higher tissue levels (Table 2). The latter observation suggests that PTU-fed mice are less sensitive to given tissue concentrations of drug (even while destroying the drug more rapidly). Timeras and Woodbury[11] reported that PTU exerts a direct depressant action on the central nervous system of the rat. This seems not to be the case in the pentobarbital-injected mouse.

Thyroidectomized rats destroy pentobarbital very slowly and show a marked prolongation of sleeping time. This is probably due to their general decrease in metabolic rate; the effect may be augmented by decreased body temperature.[20]

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