14. Scott, J. F., Fraccastoro, A. P., and Taft, E. B., *J. Histochem. and Cytochem.*, 1956, v4, 1.

15. Steudel, H., and Peiser, E., *Z. Physiol. Chem.*, 1922, v120, 292.

16. Webb, J. M., and Levy, H. B., *J. Biol. Chem.*, 1955, v213, 107.

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Relationship Between Sleep, Biotransformation Rates, and Plasma Levels of Pentobarbital and Secobarbital in Animals. (23252)

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Pentobarbital (Nembutal) (5-ethyl-5-(1-methylbutyl)-barbiturate sodium) and secobarbital (Seconal) (5-allyl-5-(1-methylbutyl)-barbiturate sodium) are very similar in structure and both are classed as "short-acting" in duration of action(1). Pentobarbital has been widely studied in animals with regard to its fate and metabolism, but there is little comparable data on secobarbital. Man seems to be the only species studied(2,3) in this manner.

It was our purpose to compare these 2 barbiturates with regard to sleeping time, body or plasma levels at the time of waking and rate of destruction in mice, rabbits, and dogs given single doses (equivalent by weight) of pentobarbital and secobarbital.

Materials and methods. The molecular weights of pentobarbital and secobarbital are within 5% of each other. This slight difference would not be evident in experiments that have biological errors which are considerably larger than 5%, so equivalent doses in weight per kilo were used throughout this work. Groups of female, albino mice, weighing between 18-22 g were injected intraperitoneally with either barbiturate as the sodium salt made to a 2% solution. Sleep was recorded as the interval between the time mice remained on their back to the time they woke and rolled over. For experiments measuring both body level and sleeping time of drug, each mouse was killed by impact, on waking, the whole animal homogenized in a Waring blendor and an aliquot analyzed for barbiturate. To determine rates of destruction, separate experiments were run with groups of mice analyzed at definite time intervals after injection regardless of whether they were asleep or awake. A group of female Sprague-Dawley rats weighing between 230-330 g was injected intraperitoneally with each barbiturate and sleeping time (righting time) recorded. One week later those animals that had received pentobarbital were given secobarbital and those that had received secobarbital were given pentobarbital. This cross-over arrangement equalized the effect of time and tolerance and the relatively large animal to animal variation was minimized. This design was used in some other experiments also (see below). Only sleeping times were studied on rats. Male rabbits weighing between 2-4.8 kg were injected intravenously with a 5% solution of the sodium salt of each barbiturate. Sleeping times were recorded from the time of injection to the time they righted themselves from a side position. Blood samples were taken by heart puncture at 1 and 2 hours and at the time of awaking. They were centrifuged, and an aliquot of the plasma analyzed for the barbiturate. Preliminary experiments showed that the plasma and tissues had reached equilibrium before one hour. One experiment was set up on a cross-over design as described above with a 5-day interval between experiments. Female mongrel dogs weighing between 5 and 12 kg were injected intravenously with 3% solutions. Blood samples were drawn from leg veins at 1 and 4 hours and at waking time and the plasma analyzed. The experiment was set up in a cross-over design as described above for rats with one week intervals between experiments. Pentobarbital and secobarbital were determined by the method of

TABLE I. Comparison of Action of Pentobarbital and Secobarbital in Mice Given Varying Doses Intraperitoneally.

	Pentobarbital					Secobarbital				
Dose, mg/kg	No. of mice	Sleeping time, min.	Whole mouse content at waking, μg/g	% dose recovered	(Calculated) destruction, %/hr	No. of mice	Sleeping time, min.	Whole mouse content at waking, μg/g	% dose recovered	(Calculated) destruction, %/hr
40	10	24 ± 6*				10	24 ± 9			
50	9	20 ± 6	33 ± 4	66 ± 8	137	10	59 ± 30	24 ± 4	47 ± 7	93
60	10	74 ± 17				10	90 ± 18			
80	10	93 ± 21	29 ± 7	36 ± 9	69	9	168 ± 23	19 ± 4	23 ± 5	53
80	30				78†	30				55†
100	10	124 ± 24	28 ± 4	28 ± 4	64	10	275 ± 82	41 ± 4	31 ± 4	28
120	8	179 ± 41	27 ± 5	23 ± 4	51	10‡				

* Mean ± stand. dev. † Data from Fig. 1. ‡ Nine out of 10 died.

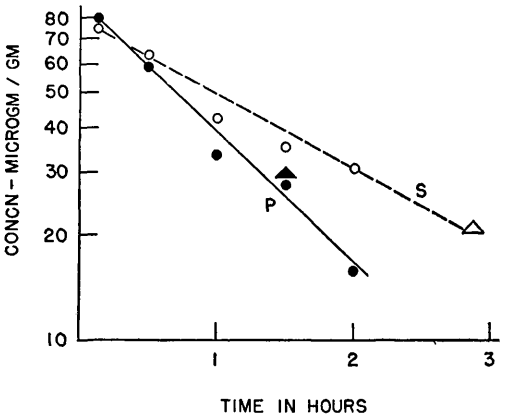

FIG. 1. Whole animal levels of pentobarbital (●——●) and secobarbital (○- - -○) in mice inj. intraper. with 80 mg/kg. Each point is the avg of 10 analyses on 10 mice. Avg time of waking and whole animal level of pentobarbital (▲) and secobarbital (△).

Brodie *et al.*(4). Recoveries of either barbiturate added to rabbit plasma or whole mice ranged between 95-97 and 93-99%, respectively. Preliminary experiments not reported here established that in the rabbit and dog, the plasma barbiturate levels decreased in the usual logarithmic straight line manner between the time intervals reported here. The rates of decrease in terms of per cent per hour were calculated from these 2 points, averaged, and the standard deviations calculated on the assumption that the rates in terms of per cent per hour were normally distributed. Plasma levels at waking time were averaged on the assumption that they were normally distributed.

Results. The data on rate of destruction of a single dose of 80 mg/kg pentobarbital and secobarbital injected intraperitoneally into mice are summarized in Fig. 1. Mice receiving pentobarbital slept a significantly shorter time (93 ± 21 (S.D.) min. *vs.* 168 ± 23 min.) ($P = < .001$) than those receiving secobarbital. Pentobarbital was destroyed at a significantly greater rate than secobarbital (78 ± 15 *vs.* 55 ± 7 (S.D.) %/hr respectively) ($P = < .001$). In another experiment it was shown that at waking time, mice receiving pentobarbital exhibited higher whole animal levels than those receiving secobarbital (29 ± 7 (S.D.) *vs.* 19 ± 4 μg/g respectively) ($P = < .01$). The brains of mice re-

TABLE II. Comparison of Pentobarbital and Secobarbital in the Rat, Rabbit and the Dog.

	Pentobarbital					Secobarbital				
Species	Dose, mg/kg	No. of animals	Sleeping time, min.	Plasma conc. at waking, μg/ml	Rate of destruction, %/hr	No. of animals	Sleeping time, min.	Plasma conc. at waking, μg/ml	Rate of destruction, %/hr
Rat	20	20*	89 ± 284			20*	77 ± 29		
	30	19*	207 ± 27			19*	204 ± 29		
Rabbit	15	10	41 ± 12	9.6 ± 1.1		5	36 ± 4	9.5 ± 1.0	
	25	15*	89 ± 19	9.6 ± 1.7		15*	104 ± 21	7.9 ± 2.0	
	25	5	114 ± 36	10.5 ± 2.0	35 ± 10	5	133 ± 44	9.0 ± 1.3	44 ± 6
Dog	20	6*	52 ± 19	14.8 ± 1.6		6*	89 ± 32	11.2 ± 1.4	
	25	6*	104 ± 38	17.2 ± 2.2	15 ± 2	6*	183 ± 46	12.3 ± 1.1	14 ± 3

* Cross-over experiment (see text for explanation). Rabbits—5 day interval between experiments. Dogs—7 day interval between experiments.
† Mean ± stand. dev.

ceiving pentobarbital were also analyzed at waking. The brain pentobarbital/g wet wt body (minus head) (pentobarbital/g wet wt) ratio of 10 mice averaged 1.01 ± 0.34 (S.D.).

Table I summarizes the data on sleeping time, calculated rate of destruction and whole animal levels of pentobarbital and secobarbital at waking time in mice given different doses of each. At the lower doses of 40 and 60 mg/kg the longer sleeping times of mice given secobarbital were not statistically significant ($P = > .05$) but at doses of 50, 80, 100 and 120 mg/kg mice slept significantly longer ($P = < .01$). Whole animal levels at awaking were relatively constant for pentobarbital regardless of dose or length of sleeping time, but secobarbital whole animal level values at waking showed a greater variation. At doses of 50 and 80 mg/kg the whole animal level at waking was significantly higher with pentobarbital than with secobarbital ($P = < .01$). However, at 100 mg/kg the situation was reversed. This high secobarbital value may be caused by the relatively longer sleeping time of 4½ hours on this high dose.

Assuming that the decline in whole animal content is logarithmic with time (as shown in Fig. 1) and that at injection 100% of the drug is in the animal one can calculate rate of destruction. On the basis of per cent per hour destroyed it is evident that rate of destruction tends to be dependent on the dose. As the dose increased the rate decreased for both barbiturates. This is unusual because similar studies of barbiturates in other species indicate that rate of destruction is independent of dose. However, at all doses studied, pentobarbital was destroyed at a more rapid rate than secobarbital.

Table II summarizes similar data on rats, rabbits, and dogs. There was no significant difference in sleeping time between these 2 barbiturates at either 20 or 30 mg/kg in rats receiving the drug intraperitoneally.

At a dose of 15 mg/kg in rabbits (I.V.) there was no significant difference in either sleeping time or plasma level at waking between these 2 drugs. However, when a larger dose of 25 mg/kg was used, rabbits slept a

shorter length of time on pentobarbital (89 ± 19 (S.D.) *vs.* 104 ± 21 min.) (P = <.01). These animals woke with slightly higher pentobarbital plasma levels than those receiving secobarbital (9.6 ± 1.7 (S.D.) *vs.* 7.9 ± 2.0 mg/g) (P = <.01). In a second experiment of 5 animals it was found that rate of destruction of pentobarbital and sleeping time again were less than that of secobarbital. Because of the small number of animals the difference in sleeping time was not statistically significant. At the dose of 15 mg/kg the brain/plasma ratio in 15 rabbits at waking time was 1.66 ± 0.2 (S.D.) for pentobarbital and 1.61 ± 0.1 for secobarbital.

At an intravenous dose of 20 mg/kg dogs slept a shorter time and woke with higher plasma levels with pentobarbital than with secobarbital, but the differences were not statistically significant (P =>.05). However, at a higher dose of 25 mg/kg the shorter sleeping time and the higher plasma levels with pentobarbital became significant (P = <.01). Rate of destruction of both of these compounds is almost identical (15 ± 2 *vs.* 14 ± 3% per hour).

Discussion. Our values for pentobarbital plasma levels at waking are essentially in agreement with those found by Goldbaum and Schack(5). At a dose of 30 mg/kg intravenously in their experiments, rabbits woke with plasma levels of 12 ± 0.4 (S.D.) μg/ml. Our results substantiate the results of these investigators in that there was no significant correlation between duration of sleeping time and plasma levels at waking in rabbits. However, there is a species difference for pentobarbital plasma level at waking. A survey of the recent literature(6) together with results of this investigation indicates that the critical plasma level at waking decreased in the following order: mouse > rat > dog > rabbit > man.

This work emphasizes the importance of comparing 2 barbiturates at more than one dose level in the mouse, dog and rabbit in order to determine potency by sleeping time response. At low doses no statistically significant differences could be demonstrated in these animals, but at higher doses a statistically significant difference became apparent. In addition, the slopes of the response curves differed in these 2 barbiturates. Thus, in addition to specifying the species studied, one must also specify the dose level studied with these 2 barbiturates.

A significant and unusual observation noted in this work was that rate of destruction of both barbiturates in mice decreased with increasing dose. Ordinarily rate of destruction of a drug is considered to simulate a first order reaction in which amount of drug destroyed is proportional to amount of unchanged drug present, and is independent of dose. A survey of the literature indicates that the problem of comparison of rates of barbiturate destruction at different doses has not been investigated to any extent. We hope to investigate this phenomenon further because of the widespread use of the mouse in problems of barbiturate sleep potentiation.

Summary. Pentobarbital and secobarbital were compared in equivalent doses in the mouse, rat, rabbit and dog. When given pentobarbital, female mice destroyed it faster, slept a shorter length of time, and woke at higher body levels than with secobarbital. Female rats showed no significant difference in sleeping times between these 2 drugs. Male rabbits and female dogs slept a shorter length of time and woke with higher plasma levels with pentobarbital than with secobarbital. There was no statistically significant difference in rate of destruction between these 2 drugs in these 2 species. In the mouse rate of destruction of both drugs was dependent on size of the dose, the higher the dose, the lower the rate of destruction. Pentobarbital and secobarbital had different dose-sleeping time response curves in mice, rabbits and dogs (but not rats).

1. Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics,* Macmillan Co., N. Y., 1955.

2. Fisher, R. S., Walker, J. T., and Plummer, C. W., *Am. J. Clin. Path.,* 1948, v18, 462.

3. Goldbaum, L. R., Fazekas, J. F., Koppanyi, T., and Shea, J., ***J. Pharm. Exp. Therap.,* 1955, v113, 23.**

4. Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M., *ibid.,* 1953, v109, 26.

5. Goldbaum, L. R., and Schack, J. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 96.
6. Richards, R. K., and Taylor, J. D., *Anesthesiology*, 1956, v17, 414.

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Desoxyribosenucleic Acid as Index of Mammary Gland Growth of Mice.*† (23253)

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Several methods have been proposed to estimate quantitatively mammary gland growth (1.2). Since the suggestion(3) that desoxyribosenucleic acid (DNA) may be constant in the somatic cells of various tissues of any species, DNA has been employed as a measurement of somatic growth and as an index of mammary gland growth in rats(4) and rabbits(5). Recently Griffith and Turner (6) have shown that DNA is constant per nucleus in mammary gland and in other somatic tissue cells of rats and mice during pregnancy. Further, it has been shown that changes in collagen content of the mammary gland are small in comparison to changes in DNA, indicating that most of DNA change during growth is due to mammary parenchymal increments(7). With the establishment of DNA as an accurate measure of mammary gland growth and a sensitive technic of determination available(8), the use of male mice was explored as assay animals for the determination of mammary gland stimulating hormones.

Methods. Male albino mice weighing 16-18 g at start of the experimental periods were used. In first experiment, groups of mice were fed a ration containing 1.23 mg diethylstilbestrol/kg ground mouse food for periods of 0, 1, 2, 3 and 4 weeks, and sacrificed after each period. In second experiment, mice were maintained on a similar diet for 4 weeks in order to insure good development of the mammary duct system(9), then divided into groups receiving graded amounts of estradiol benzoate plus progesterone subcutaneously for 10 days, using a 1:1000 ratio of the 2 substances. This particular ratio has been reported to be optimal in the production of mammary lobule-alveolar development in mice(10,11). Estradiol benzoate (0.75 μg/day) alone was injected into one group for the same length of time. Mice were sacrificed approximately 24 hours following the last injection, skinned, and ¾ of subcutaneous fascia, including mammary glands, were removed for DNA determination. The tissues were quickly frozen by placing the tissue container in an alcohol dry ice bath. The frozen tissues were then lyophilized, fat extracted in hot alcohol and then ether for 12 hours, and ground to a fine powder in a Wiley mill. DNA was extracted from 30 mg dry, fat-free tissue samples by procedure of Schneider(12) with exclusion of the fat extraction step. The tissue was extracted twice with 5 ml of cold 10% trichloroacetic acid (TCA), in a cold room held at 4°C, centrifuged and supernatant discarded. The residue was extracted with 10 ml of hot 5% TCA, and then 5 ml, for periods of 20 minutes. The supernatants were pooled, brought up to a volume of 15 ml, and DNA determined on 2 ml aliquots by the p-nitrophenylhydrazine method of Webb and Levy(8) in preference to the older diphenylamine method of Dische(13). The same purified standard DNA preparation was used throughout all experiments; however, the phosphorous content and the nitrogen content of the preparation were not checked. The remaining quarter of tissue was fixed in Bouin's fluid, washed in water, stained in Delafeld's hematoxylin, differentiated in acid-

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