has been developed for evaluation of oxytoxic drugs by the intrauterine balloon method on puerperal patients. Since the aim of ergot assay is the evaluation of the therapeutic effect of the drug, it should be helpful in establishing the validity of laboratory procedures to collaborate with clinical investigators whenever possible. Parallel assays of ergot or fractions of ergot on the human uterus and by laboratory methods should also offer additional information concerning the possible effects of one alkaloid or group of alkaloids on the action of another.

Finally, it is suggested that a step might be taken which would solve the problem of assay of galenical preparations, at least as far as the Pharmacopœia is concerned. The useful alkaloids are now available in pure form. They satisfy therapeutic requirements and the problem of assay and stability is much simpler for them than for the fluidextract of ergot. Ergonovine has undoubtedly replaced the galenical preparations to a considerable degree. A survey might supply useful information regarding the extent of such replacement. Is there any reason for retaining ergot and the fluidextract of ergot in the Pharmacopœia aside from the fact that a considerable proportion of the medical profession continue to use them because they are accustomed to do so? Probably this is sufficient reason for their retention, but it is believed that such a suggestion should at least be considered by the Revision Committee.

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Use of Sodium Pentobarbital for Repeated Anesthesia in the White Rat

*By V. Everett Kinsey**

In the present study the effects of giving sodium pentobarbital (Nembutal)[1] repeatedly to white rats will be compared with those observed previously (1, 2) for the rabbit and guinea pig.

The M. L. D. for rats for intraperitoneal injections of Nembutal has been reported as being 75 mg. per kilogram by Fitch and Tatum (3) and 120 mg. per kilogram by Carmichael (4) and Swanson and Shonle (5). Further disagreement is found in the reports concerning M. L. D. for male and female animals. None of these workers mentions a difference in response of the sexes. Barron (6) states that while the female is more susceptible to amytal than the male, there is no difference between the sexes so far as Nembutal is concerned. Holck and Kanan (7), on the other hand, state that the female rat is more sensitive than the male, as judged both by sleeping time and the M. L. D.; their assertion is confirmed by Moir (8) who found that the adult female rat is definitely less resistant than the corresponding male when the sleeping time was used as a criterion of sensitivity.

It would appear from the work of Carmichael (4), who showed that the M. L. D. in rats under nine months old was only 85–95 mg. per kilogram, as compared with 120 mg. per kilogram for rats older than this, that the comparison of M. L. D. sex differences, etc., must be made with animals of approximately the same age.

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[1] Kindly supplied by the Abbott Laboratories, North Chicago, Ill.

The question of repeated anesthesia has been studied by Moir (8), who found that when Nembutal was given repeatedly, both the male and female rats developed a tolerance to this barbiturate as measured by the sleeping time. Stanton (9) has shown that only a minor degree of tolerance is developed to the maximal effects of Nembutal upon repeated anesthesia as indicated by the struggle response of rats one hour after giving injection.

MATERIALS AND METHODS

Albino rats (Wistar strain) about three to six months old, 125–250 Gm., were kept in large wire-bottom cages. They were fed Purina Dog Chow Checkers, supplemented with greens two or three times weekly. Water was given *ad lib.* Sodium pentobarbital was weighed out accurately and dissolved in sterile salt solution (0.9 per cent NaCl), containing 10 per cent ethyl alcohol, in concentrations of 6.5 mg. per milliliter. This solution was used in all of the experiments, excepting, of course, those which were designated specifically to test the influence of the alcohol; in the latter case the alcohol was omitted from the Nembutal solution in the control series. The injections were all made intraperitoneally after first wetting the area thoroughly with 70 per cent alcohol. The period during which the righting reflex was absent was used as the criterion of the sleeping time.

EXPERIMENTAL

The effect of the 10 per cent alcohol used in making up the solution of Nembutal was checked on both male and female rats. Using a standard dose of Nembutal of 31.3 mg. per kilogram, the average sleeping time of 7 male rats which were given the Nembutal solution containing 10 per cent alcohol (0.5 Gm. of alcohol per kilogram) was 71 minutes, compared to an average sleeping time of 79 minutes for 9 animals which were given the same dose of barbiturate, but no alcohol. An identical experiment was performed with female rats. Eight rats which were injected with 31.3 mg. per kilogram of Nembutal, containing 0.5 Gm. per kilogram of alcohol, slept 232 minutes compared to an average sleeping time of 231 minutes for 11 rats which were injected with the same amount of Nembutal, but no alcohol. Similar negative results were obtained when the same quantity of alcohol was included with other doses of the barbiturate which varied from 15.9 to 62.5 mg. per kilogram. The fact that the female rats slept so much longer than the male rats will be discussed later. The author (1, 2) had found that there was no appreciable evidence of synergism between ethyl alcohol and Nembutal in the guinea pig at similar dosage levels of alcohol, but that only one-tenth as large a quantity of alcohol was needed to more than double the sleeping time of rabbits when they were given 44 mg. per kilogram of Nembutal.

The standard dose of Nembutal of 31.3 mg. per kilogram was now given to 10 male rats daily and to 10 other male rats every other day for a period of 13 days. Two groups of 10 female rats each were treated in an identical manner. The average sleeping times for the 520 anesthesias are shown in Fig. 1.

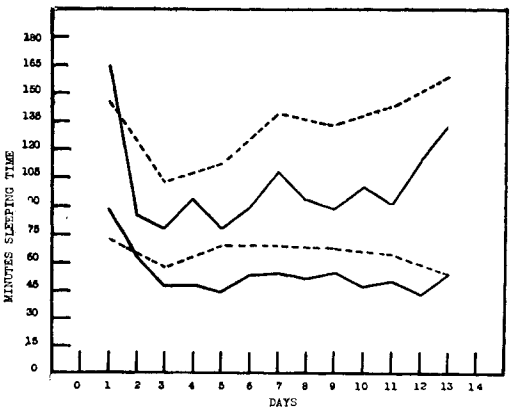

Fig. 1.—Showing the Average Sleeping Time of Rats Resulting from Intraperitoneal Injections of 31.3 mg. per Kg. of Nembutal. Top broken line—10 Female Rats Injected on Alternate Days. Top solid line—10 Female Rats Injected Daily. Lower broken line—10 Male Rats Injected on Alternate Days. Lower solid line—10 Male Rats Injected Daily.

From the two upper curves which represent the female rats, it may be seen that these rats sleep considerably longer than do the male rats, whether the injections are administered daily (solid line) or every other day. Certain it appears, too, that daily injections of Nembutal produce some tolerance to the drug in both sexes. Not so certain, however, is the repeated effect of the anesthesia when it is given every other day, since the sleeping times appear to be only slightly less, on the average, than they were originally. In this respect, likewise, the response of the rat is similar to that of the guinea pig, but different from that of the rabbit—which seems to require at least four days to lose the tolerance developed to repeated injections of Nembutal.

Because many investigators have been unable to find a difference in effect between the sexes to Nembutal, and because neither the guinea pig nor the rabbit seems to exhibit a difference in the response of the sexes, it was thought advisable to recheck the findings presented above. This was carried out on two additional groups of rats. Since it is possible that the diet may be responsible at least in part for the differences observed, one of the group (4 males and 5 females) was

kept on a synthetic diet[2] from the time of weaning until they were given the repeated anesthesias at the age of 14 weeks. The other group (6 males) was given the usual laboratory diet.

The average sleeping times for all of the males and females, injected daily and on alternate days, have been assembled in Table I. Notwithstanding the large variations in the individual sleeping times, it is evident that in all cases the female animals would sleep almost twice as long as the males when given the same dose of Nembutal. These observations confirm and extend the work of Moir (8) and Holck and Kanan (7) who had found previously that the female rat was more susceptible than the male.

Having investigated the response of the male and female rat to a particular dose of Nembutal it was decided to continue the study using increasingly larger doses. Thus it should be possible ultimately to find whether the female succumbed to a lesser dose than the male, as stated by Holck and Kanan (7), and perhaps to obtain some indication whether the M. L. D. is 75 mg. or 120 mg. per kilogram, as

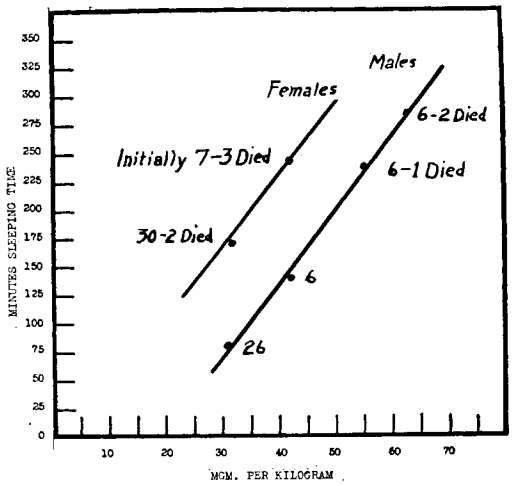

Fig. 2.—Showing the Sleeping Time Resulting from Increasing Doses of Nembutal.

Table I.—Showing the Length of Sleeping Time of Male and Female Adult Rats Following Repeated Intraperitoneal Injections of Nembutal (31.3 mg./Kg.)

Males					Females				
Number of Animals	Number of Injections per Animal	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Animal, Min.	Range of Individual Sleeping Time of Each Animal, Min.	Number of Animals	Number of Injections per Animal	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Animal, Min.	Range of Individual Sleeping Time of Each Animal, Min.
Injected Daily									
10	13	54.0	46–73	24–132[a]	10	13	101	80–124	44–201[a]
3	19	39.0	38–40	20–70					
4[b]	14	43.5	41–45	21–86					
Mean average 48.8 for 243 anesthesias					Mean average 101 for 130 anesthesias				
Injected Every Other Day									
10	7	63.8	61–71	38–102	10	7	133	106–164	61–210[a]
3	10	53.6	51–56	39–76	3[b]	7	91	77–101	31–197[a]
Mean average 61.5 for 100 anesthesias					Mean average 119 for 105 anesthesias				

[a] First day of injection.
[b] Synthetic diet (complete).

variously found by the workers cited in the first part of this paper.

The four doses of sodium pentobarbital selected for study were 31.3, 42, 55 and 62.5 mg. per kilogram. All of them were made up in 10 per cent alcoholic saline (0.9 per cent NaCl) solution. The results of these single injections appear in Fig. 2. There it may be seen that the sleeping time appears to increase directly with the dose, but, as in the case of guinea pigs, the dose must exceed a threshold value, which in the present case amounts to approximately 10 mg. per kilogram for the female and 20 mg. for the male. That the female is more susceptible than the male is further indicated by the fact that at a dose of 55 mg. per kilogram and above (with one exception at 62.5 mg. per kilogram) all of the female rats died, while only three out of twelve male rats died when given these doses. Finally, a M. L. D. value for male rats of somewhere near 75 mg. per kilogram is indicated (*cf.* Fitch and Tatum (3)) although too few animals were used at the higher dose levels to establish the M. L. D. precisely.

Moir (8) found that castrating rats increased their susceptibility to Nembutal as measured by sleeping time. The castrated animal, however, was not as susceptible as the female, whence Moir concludes that some factor other than the male gonad is responsible for male rats being less susceptible to the hypnotic effect of sodium pentobarbital than females. Incidently Barron (6) found that castrating rats increased their chance of dying when given minimal lethal doses of sodium amytal.

As Moir inferred, it would appear that some secretion of the testis decreases the susceptibility of rats to Nembutal. To establish this idea a little more fully, 20 female rats were divided into two equal-sized groups. The first group was given 0.1 mg. of testosterone propionate subcutaneously every day for ten days while no treatment was given the second group of rats. At the end of this time both groups

[2] Casein 18%, salt mixture 4%, butter fat 8%, cod liver oil 2%, cornstarch 68%, tiki-tiki extract from 250 Gm. of tiki-tiki, and 40 gamma per day of riboflavin.

were injected with 31.3 mg. per kilogram of Nembutal. The average sleeping times were 129 minutes for the treated rats and 230 minutes for the control rats, respectively (Table II).

Table II.—Showing the Length of Sleeping Time of Adult Female Rats Which Previous to Intraperitoneal Nembutal Injections (31.3 mg./Kg.) Received 10 Daily Subcutaneous Injections of Testosterone Propionate (1.0 mg. Total)

Received Testosterone		Controls	
Rat No.	Sleeping Time, Min.	Rat No.	Sleeping Time, Min.
1	109	6	284
2	124	7	202
3	29	8	235
4	132	9	274
5	234	10	234
11	126	14	239
12	89	15	121
13	137	18	258
16	178	19	220
20	Died		
	Average 129		Average 230

The outcome of this experiment suggests rather strongly that the testosterone does indeed confer some degree of resistance to Nembutal upon the animal receiving it. It should be noted, however, that the average sleeping time of the female rats given testosterone propionate still exceeds somewhat the average sleeping time (80 minutes) of a comparable series of male rats. This agrees with the result of Moir who found, it will be recalled, that the sleeping time of castrated rats was still not so long as that of comparable female rats.

While larger doses of testosterone propionate might still further lower the sleeping time of female rats, it seems that a hormone secreted by the ovaries might account for the fact that the female rats treated with testosterone are still somewhat more susceptible to Nembutal than are the males. To check this point 20 uniform young (150–215 Gm.) female rats were spayed, and after two weeks they were given 31.3 mg. per kilogram of Nembutal. Twenty other female rats similar in every way but unspayed, were used for controls. (The same rats were then used for work reported in Fig. 1.) Table III shows that the average sleeping times of the

Table III.—Showing the Effect of Spaying on the Sleeping Time of Rats When Anesthetized with 31.3 Mg. per Kilogram of Nembutal

No. of Rats	Average Sleeping Time, Min.	Range of Sleeping Time, Min.
	Controls	
20	156	110–210
	Spayed Rats	
20	98	70–137

spayed animals were reduced quite appreciably. It would be of interest to give testosterone propionate to spayed rats and determine whether all differences between the sleeping times of male and female rats as a result of Nembutal injections would then disappear.

In conclusion it should be emphasized that when animals are anesthetized with barbiturates, wide variations occur in the sleeping times of individual animals; and also that even when every known precaution is taken to control all variables, discrepancies may nevertheless occur. This should be borne in mind when interpreting any of the data reported above, obtained from numbers which may sometimes be statistically inadequate.

CONCLUSIONS

1. It has been shown that ethyl alcohol in concentrations of 0.5 Gm. per kilogram does not potentiate the action of varying doses of Nembutal in white rats as measured by the increased sleeping time.

2. As a result of administering 31.3 mg. per kilogram of Nembutal repeatedly to rats, the sleeping time decreases abruptly after the first dose when the injections are given at daily intervals. When the frequency of anesthesia is decreased to every other day, the decrease in sleeping time is not so noticeable.

3. Female rats sleep about twice as long as male rats when given either single or repeated injections of Nembutal.

4. Following the injection of 1 mg. of testosterone propionate subcutaneously into female rats, the average sleeping time resulting from a dose of 31.3 mg. per kilogram of Nembutal is reduced from 230 to 129 minutes.

5. Spayed rats seem to sleep only about two-thirds as long as unspayed controls.

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"Any problem which is faced with complete honesty vanishes."—

NINA WILCOX PUTNAM.