The Use of Sodium Pentobarbital for Repeated Anesthesia in the Guinea Pig

*By V. Everett Kinsey**

The first paper of this series (1) dealt with the use of sodium pentobarbital (Nembutal)[1] for repeated anesthesia in the rabbit. The present study will describe the problems which arise from the use of this drug for repeated anesthesia in the guinea pig. Rather oddly, there have been few studies of the use of sodium pentobarbital for this animal. Carmichael and Posey (2) report that, after six bi-weekly intraperitoneal injections of 15 mg. per Kg. of Nembutal, guinea pigs showed a definite tolerance to approximately 18 mg. per Kg. of this drug as measured by the sleeping time. Daily injections (period not given) produced tolerance much faster, but in no case was the M. F. D. (minimum fatal dose, *i. e.*, amount killing 90 per cent of the guinea pigs) increased as a result of previous injection. These authors (3) also studied the M. L. D. and found it to vary from 45 mg. per Kg. for 200-Gm. guinea pigs to 60 mg. per Kg. for 800-Gm. guinea pigs.

MATERIALS AND METHODS

Young guinea pigs were kept in groups of five in large wire-bottom cages. Their diet consisted of oats, bran and hay daily with greens from two to four times weekly, and some butterfat and milk powder once a week. While the Nembutal solution was but one-tenth so concentrated (6.5 mg. per ml. of 0.9 per cent NaCl containing 10 per cent ethyl alcohol) as that used in the previous study (1), the procedure for administering the intraperitoneal injections to the guinea pigs was identical with that used for the rabbits. In view of the variation of response with the weight all comparative studies were made with animals of the same weight group. The sleeping time was taken as the period during which the righting reflex was lost.

EXPERIMENTAL

In view of the fact that a possible synergistic effect of the alcohol, used in making up the solution of Nembutal, would materially alter the interpretation of the results to be reported below, the influence of the alcohol was first investigated. The sleeping time of 8 guinea pigs, given 15.6 mg. per Kg. of Nembutal in a solution containing 10 per cent alcohol (0.2 Gm. per Kg.), averaged 86 minutes, as compared to 88 minutes in a control group of 8 animals which received the same dose of Nembutal but no alcohol. None of these animals was injected before.

The above finding was confirmed by the following experiment: 40 small (200–300 gram) guinea pigs, which had not been injected previously, were divided into 8 equal-sized groups. Each group of animals was then injected with 15.6 mg. per Kg. of Nembutal, dissolved in 0.9 per cent NaCl and increasing amounts of alcohol. It may be seen from Fig. 1, where the average sleeping times resulting from these injections are plotted, that the sleeping time does not appear to be increased appreciably until at least 0.4 Gm. per Kg. of alcohol is given simultaneously with the Nembutal. This is twice the amount of alcohol given in any of the experiments which are to be described later.

It is of interest to compare the above results with the initial, greatly increased, sleeping time of the rabbit (1) with doses of alcohol as low as 0.055 Gm. per Kg.

It was observed, however, that considerable tolerance was established in the rabbit after one injection of the alcoholic Nembutal. Since some potentiation was observed above 0.5 Gm. per Kg.

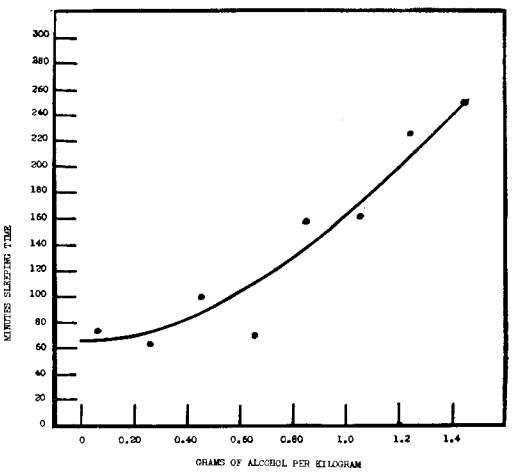

Fig. 1.—Showing the average sleeping time of 8 groups of 5 guinea pigs each, following an intraperitoneal injection of 15.6 mg. per Kg. of Nembutal and increasing quantities of ethyl alcohol.

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

of alcohol in the guinea pig it was decided to find whether the guinea pig, too, would exhibit tolerance when given a second dose of the Nembutal and alcohol. Accordingly, the experiment described above with the 8 groups of 5 animals each was repeated exactly after an interval of one week. The sleeping times for the 8 doses of alcohol were then found to be 108, 112, 108, 123, 121, 137, 251 and 243 minutes, respectively. These data indicate that no tolerance to the alcohol is evident, at least following a week's rest period, and, furthermore, they confirm the results of the first injections, *viz.*, that over 0.4 Gm. of alcohol per Kg. is necessary before any synergism is observed between the alcohol and Nembutal.

A standard dose (15.6 mg. per Kg.) of Nembutal was now given to 6 guinea pigs daily, and to 6 other guinea pigs on alternate days for a period of 19 days. The sleeping times were recorded. (See curves, left-hand part of Fig. 2.) Then because all of the guinea pigs used for long-term studies (described below) were fed the Sherman, La Mer and Campbell (4) vitamin C free diet, supplemented with pipetted feedings of ascorbic acid, the above results were compared to those obtained by repeating the experiment on the same animals two weeks later. During this interval the animals were placed on the special diet. (See curves, right-hand part of Fig. 2.) At the start of the second series of Nembutal injections, the diet was supplemented each day with a maintenance dose of 0.5 mg. of ascorbic acid for each guinea pig.

The solid lines in Fig. 2 refer to the group of animals receiving injections daily, while the dotted lines refer to the group which were injected on alternate days. Like rabbits, following the first several anesthesias, the guinea pigs exhibit an appreciable amount of tolerance which depends upon the frequency of administration. (It will be shown later, however, that when anesthesias are repeated over sufficiently long periods of time the guinea pigs increase significantly in weight, their susceptibility to Nembutal again increases and less drug is required to make them sleep for a given period.) A comparison of the right- and left-hand curves in Fig. 2 shows that the special diet, containing maintenance levels of ascorbic acid, appears to increase the sleeping times slightly; however, more animals would be required to establish the significance of this effect.

Having determined the length of time guinea pigs would sleep as a result of repeated injections of a constant dose of Nembutal, the dosage was next varied in such a way that the length of sleeping time was kept as nearly constant as possible. A period of two hours was chosen for this period of anesthesia.

Two groups of guinea pigs, *A* and *B*, containing 6 and 4 animals, respectively, were maintained on the Sherman diet supplemented with 0.5 mg. of ascorbic acid daily. These animals were injected with Nembutal every other day, excepting Saturday and Sunday, over a period of about 6 months. Throughout this period 4 of the animals of Group *A* died of respiratory collapse at the time of injection, while all of those of Group *B* survived.

The dose required each day to produce anesthesia for the 2-hour period is shown in Fig. 3. Because it was obviously impossible to produce anesthesia for

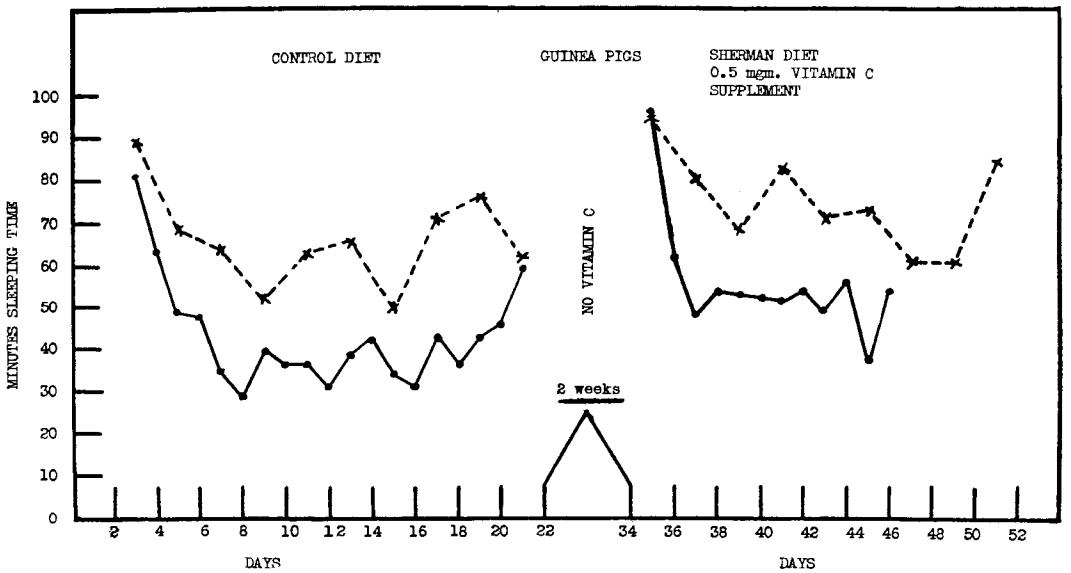

Fig. 2.—Curves on the left represent the average sleeping times of 6 guinea pigs injected daily (solid line) and 6 guinea pigs injected on alternate days (broken line) with 15.6 mg. per Kg. of Nembutal while maintaining the animals on a completely adequate diet. Curves on the right represent the same animals, treated in the same manner, but maintained on the Sherman diet supplemented with 0.5 mg. of ascorbic acid per animal per day. See text for explanation.

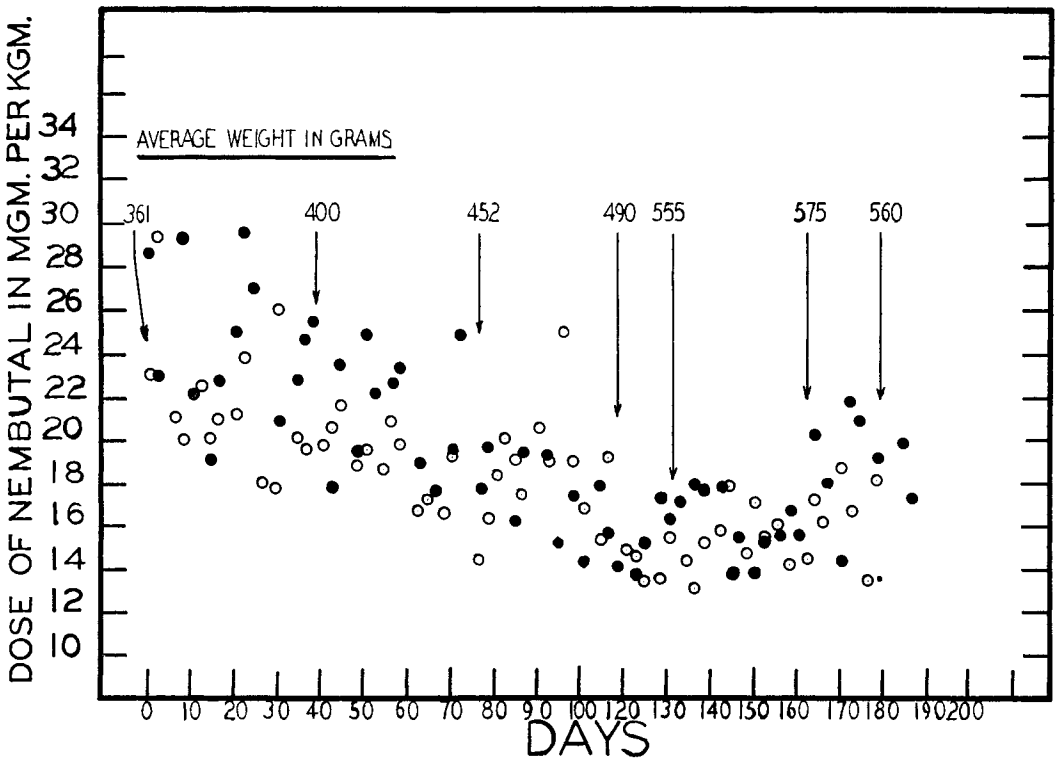

Fig. 3.—Showing the dose of Nembutal required to produce repeated anesthesia in guinea pigs for 120 minutes. Group A Open Circle—6 animals; Group B Solid Circle—4 animals.

precisely the 2-hour test period a small correction had to be made in the dose to adjust it up or down depending upon whether the average sleeping time was slightly above or below the 120 minutes. For example, if the average sleeping time on any particular day was 110 minutes instead of the desired 120 minutes the additional amount of Nembutal which would have been required to increase the sleeping time 10 more minutes was estimated from data which will be presented later in this paper (Fig. 4).

Figure 3 shows that the dose necessary to produce sleep for 120 minutes appears to decrease progressively throughout practically all of the experimental period, *i. e.*, the animals gradually became more, rather than less, susceptible to the drug. Of interest is the very close inverse relationship between the dose and the average weight of the animals. It will be seen from the figure that while the weight increases regularly up to the 150th day, the dose decreases, but following a rather abrupt increase to a nearly constant weight, due in part to the death of a 360 Gm. animal, the dose, too, remains practically constant. Thus it would seem that not only does the M. L. D. as noted by Carmichael and Posey (3) decrease with heavier (or older) guinea pigs but so does the sleeping time. It should be noted that no difference in the results would have been obtained if the dose had been based on area of the animal as suggested by Moore (5) instead of the weight. It would appear that both dosage on a weight basis and dosage on an area basis must still be considered arbitrary in the case of Nembutal and guinea pigs. In contrast when working with rabbits the ratio of the dosage to the sleeping time was found to remain constant when Nembutal was given repeatedly over a period of almost a year.

Having studied the dosage required to produce a particular sleeping time, *i. e.*, 2 hours, the response to varying doses was next investigated. The doses selected were 15.6, 25, 31.3 and 37.5 mg. per Kg. In a few instances one guinea pig would be used a second time, but a rest period of at least a week was allowed between tests. (No differences in sleeping times were detected in such cases.) The average and range of sleeping times for the animals in these experiments appear under "Single Dose" in Table I and graphically in Fig. 4 where it may be seen that the sleeping time increases linearly with the dose when a threshold amount of about 10 mg. per Kg. is exceeded. The average weight of the guinea pigs was 350 Gm. with an extreme range of 300 to 600 Gm. Of the 51 animals receiving the first dose (15.6 mg. per Kg.) the sleeping time was slightly lower for the 33 weighing less than 350 Gm. than for the 18 which weighed more than this amount. Considering the wide individual variations encountered, these differences (86 *versus* 96 minutes) may not be significant.

Table I.—Comparison Between the Lengths of Sleeping Time of Guinea Pig When Injected with as Single Dose of Nembutal and When the Same Amount Is Given in Two Separate Injections

No. of Guinea Pigs	No. of Groups	Dose (Injections) First + Second = Total mg./Kg.	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Group, Min.	Range of Individual Sleeping Time, Min.
		Single Dose			
51	7	15.6	89.4	75–115	35–155
25	4	25	201	193–206	95–338
6	1	31.3	286	...	195–332
21	4	37.5	352	336–375	244–455
		Double Dose			
10	2	15.6 + 9.4 = 25	239	229–246	181–285
6	1	15.7 + 15.6 = 31.3	269	...	220–305
10	2	15.6 + 21.9 = 37.5	365	344–385	304–457
9	2	25 + 12.5 = 37.5	365	356–375	273–458

Table II.—Showing the Lengths of Sleeping Time of Male and Female Guinea Pigs When Injected Intraperitoneally with 15.6 mg. per Kg. of Nembutal

Sex	Number of Guinea Pigs	Average Sleeping Time, Min.	Range of Sleeping Time, Min.
Males	25	92.4	35–190
Females	21	84.0	35–130

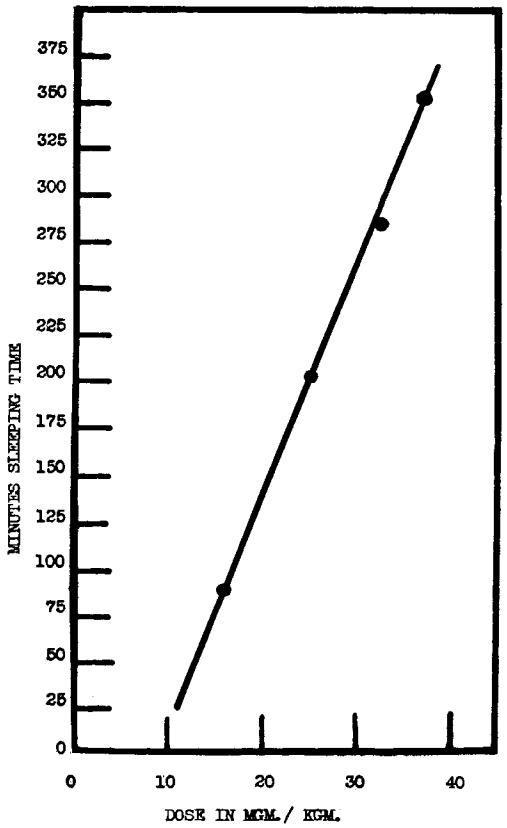

Fig. 4.—Showing the sleeping time resulting from increasing doses of Nembutal.

It had been found (1), particularly in the case of rabbits, that when longer sleeping times were desired it was necessary for the safety of the animals to give a second and sometimes a third dose of Nembutal. This raised the question of the comparative sleeping time of divided doses, as against giving the total amount or drug in a single injection. The guinea pig provides an excellent test animal for this purpose because of the wide range between the anesthetic and lethal dose. The three higher doses used previously, 25, 31.3 and 37.5 mg. per Kg., respectively, were each divided into two doses. The first injection was given and, immediately after the animal regained consciousness, the second dose was administered. It will be seen from the lower half of Table I that the total sleeping time is about the same whether the drug is given all at once or in two doses.

Lastly, the response of male and female guinea pigs, as judged by the sleeping time, was tested. A dose of 15.6 mg. per Kg. of Nembutal was given to 25 male and 21 female animals. The average sleeping times and range of the sleeping times given in Table II show that there is little difference between the two sexes. The average weights of the two groups were 338 and 366 Gm. for male and female animals, respectively.

CONCLUSIONS

1. It has been shown that ethyl alcohol does not potentiate the action of Nembutal in guinea pigs as measured by increased sleeping time, unless given in amounts in excess of 0.4 Gm. per Kg. with doses of Nembutal of 15.6 mg. per Kg.

2. The sleeping time of guinea pigs subjected to repeated anesthesia with 15.6 mg. per Kg. of Nembutal decreases for the first several daily injections. Similarly treated guinea pigs maintained on a low level of ascorbic acid appear to sleep about 10 per cent longer than those given an adequate supply of greens.

3. When anesthesia of 2 hours' duration is produced every other day over a six-month period by giving intraperitoneal in-

jections of Nembutal, the susceptibility of the animals to this drug gradually increases. This increase appears to be associated with an increase in the weight of the animals.

4. The sex of the guinea pig did not appear to alter the sleeping time following single injections of Nembutal.

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Potassium Bismuth Saccharate. II. Toxicity, Absorption and Distribution of Bismuth Following Intramuscular Injection*

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The preparation and chemical properties of a water-soluble potassium bismuthyl saccharate have been given in a previous report from this laboratory (1). This paper presents a study of the absorption, distribution, excretion and toxicity of a preparation containing this compound.[1]

EXPERIMENTAL

The absorption, distribution and excretion in rabbits was determined after a single intragluteal injection of doses equivalent to 5 mg. and 3 mg. of bismuth per Kg. body weight into two groups of six animals each. These doses were as small as the precision of the analytical method would permit for subsequent determination of the distribution of the bismuth in the various organs. One, three and five days after the injection, two animals in each group were sacrificed by exsanguination and the organs immediately removed for assay. The excretion was determined by collecting the urine and feces separately after each twenty-four-hour interval.

Quantitative determinations of the bismuth content of the biological material were made by the iodide method for amounts of the metal in excess of fifty micrograms (2), and by the dithizone method for smaller concentrations (3).

Table I presents the data on total excretion of bismuth in terms of the per cent of the amount injected.

Table I.—Excretion in Per Cent of Amount of Bismuth Injected

Dose, mg./Kg.	Rabbit No.	Excretion Time, Days	Urine	Feces	Total
5	1	1	6.0	0.7	6.7
	2		0.8	0.1	0.9
	3	3	1.2	0.5	1.7
	4		8.1	4.7	12.8
	5	5	11.6	3.7	15.3
	6		17.4	6.5	23.9
3	7	1	1.2	0.1	1.3
	8		0.3	0.1	0.4
	9	3	13.1	0.2	15.1
	10		15.6	2.0	17.6
	11	5	16.1	2.6	18.7
	12		23.3	4.1	27.4

It is seen that with the larger dose of bismuth about one-fifth of the amount injected is excreted within the five-day period with the peak of the excretion occurring after the first three days. With the smaller dose a disproportionately smaller amount was found in the excreta. The daily variation in the amount found was roughly proportional to the quantity of excreta but individual animals showed no constant relationship between the amount of bismuth excreted and the time after injection.

Table II shows the distribution of the bismuth in the organs of the injected animals. In addition the last column gives the total recovery of bismuth in terms of the per cent of the injected quantity.

The toxicity of potassium bismuthyl saccharate for white rats by both intravenous and intramuscular injection is shown in Table III.

Sections of the kidneys of the animals receiving a fatal dose of the bismuth compound showed widespread pathology. The rapidly fatal doses produced desquamation of the epithelium of the convoluted tubules, dilatation of the tubules and abundant albuminous casts. Animals living longer than one week but subsequently succumbing to the metal showed, in addition to the above changes, focal areas of necrosis of the kidney parenchyma and more or less extensive calcification of the tubular epithelium. The animals receiving a sub-lethal dose showed, after three weeks' observation, cloudy swelling and some desquamation of the tubular epithelium.

* From the Research Laboratories of George A. Breon & Co.

[1] Sacbimuth—a solution containing 50 mg. potassium bismuthyl saccharate per cc. (equivalent to 25 mg. bismuth), 25 per cent sucrose and 2 per cent benzyl alcohol.