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Studies on Barbiturates XXIV

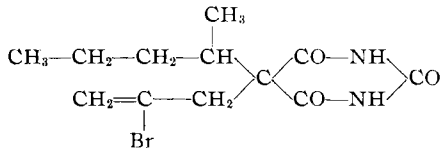
Pharmacology of Secondary Amyl-Beta-Bromallyl Barbituric Acid*

By Lloyd W. Hazleton, Theodore Koppanyi and Charles R. Linegar†

This study is the survey of the pharmacological actions and toxic properties of a relatively new barbiturate; 5-sec. amyl-5-beta-bromallyl barbituric acid, named "Sigmodal" by its manufacturers, Messrs. Riedel-de Haen, Inc.

CHEMISTRY

This compound is a white crystalline powder of bitter taste with the following structural formula:



($C_{12}H_{17}O_3N_2Br$)

It is difficultly soluble in water and chloroform, soluble in ether and alcohol, and melts at 161° to 163° C. The sodium salt is soluble in water, more difficultly soluble in alcohol and practically insoluble in ether. The aqueous solution is unstable and should not be used after a few days of standing. A 10 per cent solution of sodium sec. amyl bromallyl barbiturate in an aqueous vehicle, each 100 cc. of which contains 10 Gm. of glycerine, 10 Gm. of antipyrine and 10 cc. of alcohol as stabilizing agents, is recommended by the manufacturers for rectal administration.

Sigmodal gives in chloroform solution the characteristic blue color in the Koppanyi test for barbiturates (1), (2), (3), (4). There is an apparent decrease of sensitivity to the test owing to the high molecular weight of the compound (5), the color being about 60 per cent that of barbital. The readings for the test are made as for barbital and then corrected by multiplying the results by 1.7.

The technique used in estimating sigmodal in the urine, etc., is the same as that for barbital except that ether is used for extraction. If chloroform is used for extraction, at least three extractions should be made because of the low solubility of this compound in chloroform. The ether or chloroform extracts are evaporated to dryness and the residues taken up in chloroform for the colorimetric estimations. It is possible to make a 0.2 per cent solution of the acid in chloroform which is sufficient for the needs of the test.

EXPERIMENTAL

Sigmodal was used for intravenous injections in a 2 per cent aqueous solution of the sodium salt, and

* This substance was prepared in the laboratories of Riedel-de Haen, Inc., and a grant by this company partially defrayed the expense of this study.

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occasionally, for prolonged intravenous infusions in a 0.2 per cent solution. The 10 per cent solution of the sodium salt in water, with 10 per cent each of glycerine, antipyrine and alcohol as supplied by the manufacturers was used for rectal administration.

TOXICITY

The average (50 per cent) fatal dose was determined intravenously in rabbits and dogs, and rectally in rabbits. The results are shown in Tables I, II and III.

dose of 30 mg. failed to kill any of six animals. The data on the rectal administration of sigmodal to dogs are too incomplete to warrant a general statement. The cause of death is always arrest of respiration, the heart continuing to beat vigorously for several minutes after respiration has ceased. In rabbits the average intravenous fatal dose caused death in about eight minutes, and the rectal fatal dose in about twenty minutes following administration of sigmodal sodium. In dogs death following intravenous doses occurs more rapidly.

Table I.—Results of Intravenous Administration of Sigmodal Sodium in Rabbits

Dose Mg./Kg.	Number of Auimals Total	Lived	Died	Recovery[a] Time Minimum Minutes	Maximum Minutes	Average Minutes	Time of Death Minutes
10	12	12	0	...	...	0	.....
15[b]	6	6	0	...	...	30	.....
20	3	3	0	53	80	64	.....
25	5	5	0	45	72	67	.....
30	7	7	0	64	122	88	.....
40	11	6	5	105	125	118	3 to 15
45	5	0	5	...	...	...	2 to 8

[a] Ability to maintain upright posture unassisted.
[b] Loss of motor coördination; righting reflex absent in four animals.

Table II.—Results of Rectal Administration of Sigmodal Sodium in Rabbits

Dose Mg./Kg.	Number of Animals Total	Lived	Died	Average Recovery Time Minutes	Time of Death Miuutes
50[a]	7	7	0	98	.....
60	10	9	1	158	40
80	2	2	0	240	.....
90	5	3	2	294	11 and 34
100	2	0	2	...	22 and 36

[a] No narcosis in two animals.

Table III.—Results of Intravenous Administration of Sigmodal Sodium in Dogs

Dose Mg./Kg.	Number of Animals Total	Lived	Died	Average Recovery Time Hours	Time of Death Minutes	Remarks
20	2	2	0	1.8	.....	Righting reflexes present, incomplete relaxation, poor analgesia
25	2	2	0	...	.....	Good anesthesia, surgery performed
30	6	6	0	5	.....	Good anesthesia, surgery performed
35	2	1	1	About 6	3	
40	3	0	3	...	Almost immediate respiratory stoppage	.....

In rabbits the 50 per cent intravenous fatal dose is approximately 40 mg. per Kg.,[1] whereas all animals receiving 45 mg. died. The 50 per cent rectal fatal dose in rabbits is about 90 mg., *i. e.*, slightly more than twice the intravenous dose, while 100 mg. produced 100 per cent fatalities. In the dog the average intravenous fatal dose is about 35 mg. A

[1] All doses are expressed in terms of mg. of sigmodal sodium per Kg. of body weight. To avoid repetition the words "per kilogram of body weight" will hereafter be omitted.

EXCRETION

The excretion of sigmodal in the urine was determined in rabbits and dogs. Five rabbits (No. 7, 8, 9, 10 and 11 of Table IV) showed during the first six hours the following percentage excretion in the urine: 7, 17, 3.8, 4.5 and 5.6 per cent respectively.

In two dogs a single dose of 30 mg. of sigmodal sodium was given intravenously and the barbiturate content of the urine was determined during a period of 48 hours. Only traces of the drug were excreted during the second 24 hour period. In the first dog a

total excretion of 20 per cent and in the second, of 21.8 per cent was observed. These figures represent the results obtained by using the Koppanyi colorimetric test for barbiturates, modified as described in the section on Chemistry.

ESSENTIAL ELIMINATION

The persistence of action of sigmodal was studied in rabbits by two different methods:

In the first method fractions of the intravenous 50 per cent fatal dose were injected at half-hourly and hourly intervals until death occurred. The difference between the fatal dose determined in this way and that determined by a single injection was taken to represent approximately the amount of drug essentially eliminated during the period between the administration of the first fraction and the death of the animal.

elimination of the compound was computed by the following formula:

$$\frac{\text{50 per cent fatal dose—maximum dose of hypnotic permitting normal motor activity}}{\text{sleeping time (hours)}} = \text{essential elimination in milligrams (per kilogram) per hour.}$$

Table IV shows the results arrived at by the first method. Five rabbits were given 15 mg. of sigmodal sodium intravenously every half-hour, five rabbits 10 mg. every half-hour, and six rabbits 10 mg. every hour. Two rabbits showing little if any elimination, presumably exceptional or sick animals, were not included in the table. The animals receiving 10 mg. per hour showed no visible signs of accumulation, and after seven doses they were apparently in the same condition as after the first dose,

Table IV.—The Essential Elimination of Sigmodal Sodium after Repeated Intravenous Administration of Fractions of the Fifty Per Cent Fatal Dose in Rabbits

Rabbit Number	Single Dose, Mg./Kg.	Number of Doses	Interval between Doses, Minutes	Results[a]	Total Dose Given, Mg./Kg.	Total Dose in Percentage of 50 Per Cent Fatal Dose, Per Cent	Duration of Experiment, Minutes	Percentage Essential Elimination of 50 Per Cent Fatal Dose, Per Cent
1	10	7	60	No narcosis[b]	70	175	...	.....
2	10	7	60	No narcosis[b]	70	175	...	.....
3	10	7	60	No narcosis[b]	70	175	...	.....
4	10	7	60	No narcosis[b]	70	175	...	.....
5	10	7	60	No narcosis[b]	70	175	...	.....
6	10	7	60	No narcosis[b]	70	175	...	.....
	Average							> 25 per hour
7	10	13	30	R	130	325	...	> 225
8	10	13	30	D(7)	130	325	367	225
9	10	13	30	R	130	325	...	> 225
10	10	13	30	R	130	325	...	> 225
11	10	13	30	R	130	325	...	> 225
	Average							> 37 per hour
12	15	4	30	D(7)	60	150	97	50
13	15	7	30	D(5)	105	263	178	163
14	15	6	30	D(10)	90	225	160	125
15	15	7	30	D(2)	105	263	179	163
16	15	4	30	D(7)	60	150	97	50
	Average						142	110
								47 per hour

[a] D = Death; R = Recovery; Interval in minutes between last dose and death in parenthesis.
[b] No determinable accumulation from hourly doses of 10 mg.

The second method is a new one which may be described briefly as follows: The maximum dose which permitted the animals to maintain normal posture unsupported and to retain approximately normal motor activity was determined. This dose for sigmodal sodium was found to be about 10 mg. intravenously, which is 25 per cent of the 50 per cent intravenous fatal dose as determined by a single injection. After ascertaining the average sleeping time of all survivors from the average fatal dose, *i. e.*, until they regained the stage in which normal body posture was again maintained, the essential

therefore, they eliminated more than 25 per cent of the fatal dose per hour. Of the rabbits receiving 10 mg. every half-hour, only one died after thirteen doses; the others showed deep 4+ anesthesia but were found to be completely recovered the next morning. Therefore, they eliminated 225 per cent of the average fatal dose or more during a period of about six hours, or more than 37 per cent per hour. The rabbits receiving 15 mg. every half-hour eliminated on the average a little over one fatal dose during a period of two hours and twenty-two minutes or 47 per cent per hour. All these re-

sults seem to indicate that rabbits are capable of eliminating about 45 per cent of the fatal dose, or 18 mg. (per Kg.) per hour.

In determining the essential elimination by the second method, use is made of certain data shown in Table I. The 50 per cent fatal dose was 40 mg., the average sleeping time of the survivors from this dose was 118 minutes (2 hours), and the maximum dose not interfering with body posture and motor activity was 10 mg. Thus the essential elimination may be determined by substituting in the above formula: $\frac{40 - 10}{2} = 15$ mg. (per Kg.) per hour, a figure which agrees fairly closely with the figure obtained by the first method.

ACTION ON THE CENTRAL NERVOUS SYSTEM

Sigmodal sodium, in appropriate doses, produces incoördination, sleep, motor paralysis and anesthesia. Complete sensory paralysis is seen only in dogs with doses from 30 to 35 mg. In rabbits, even amounts approaching the fatal dose do not produce

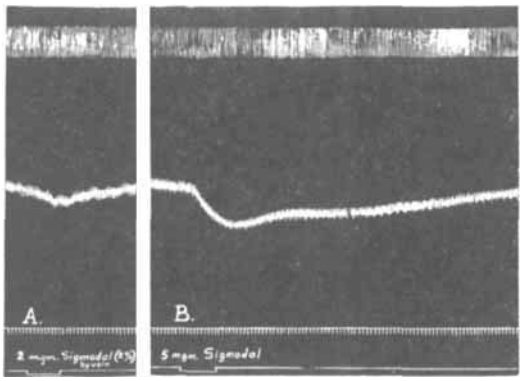

Fig. 1.—Dog, Male, Weight 6.7 Kg. Ether Anesthesia. Upper line respiration, second line blood pressure from the common carotid artery, third line base line. Time = 1.7 seconds. A. Intravenous injection of 2 mg. sigmodal sodium; B. Intravenous injection of 5 mg. sigmodal sodium. Note absence of effect on respiration.

corneal anesthesia or loss of response when the tail is pinched, or when intravenous injections are made into the ear; also the superficial tendon reflexes are retained. In rabbits the effects of 10, 15, 20, 25, 30, 40 and 45 mg. given intravenously and 40, 50, 80, 90 and 100 mg. administered rectally were studied. The rabbits receiving the 10 mg. intravenous dose retained their normal posture and were able to hop around with no, or only a slight, motor incoördination. With 15 mg. doses the righting reflexes were retained but motor coördination was lost. With 20 mg. doses the animals lost their righting reflexes and assumed a lateral position, but the muscular relaxation was very incomplete and the analgesia poor. With doses of 25 mg. or more, most of the animals showed complete muscular relaxation (all animals with 30 mg. or more but not all with 25 mg.), fair analgesia, frequently nystagmus, a state corresponding to Schoen's 4+ stage of anesthesia (6). Upon rectal administration the effective doses were much higher; with 50 mg. some animals retained their righting reflexes while with 60 mg. all animals showed 4+ anesthesia.

Dogs show true surgical, third stage anesthesia with loss of corneal reflex, complete analgesia and muscular relaxation. Several surgical operations were performed on dogs receiving 25 to 35 mg. of sigmodal sodium intravenously. The results from rectal administration of sigmodal sodium in dogs were not as uniform as in rabbits; in some animals 40, 50, 80 and 100 mg. produced about the same degree of depression (sleep, but not true anesthesia) while 150 mg. produced surgical anesthesia, and 250 mg. caused death in about five hours.

The duration of narcosis is relatively brief in rabbits; in dogs after comparable doses, it is at least twice as long. Sigmodal sodium is definitely a short-acting barbiturate.

The onset of action after intravenous injection is immediate, the animals often relaxing before the end of the injection. Upon rectal administration the onset of action in rabbits is very rapid, motor incoördination becoming evident in 2 to 8 minutes, while with suitable doses 4+ anesthesia occurs in less than 15 minutes.

The peripheral nervous system is apparently little affected by this drug; there is no paralysis of the cardiac vagus as determined by faradic stimulation even with fatal doses, and the chorda tympani likewise remains active. The cardiac and vasomotor responses to autonomic drugs are normal.

HEART, CIRCULATION AND RESPIRATION

In small or in moderate doses, sigmodal sodium had no effect on the rate or rhythmicity of the heart. With large doses a moderately increased pulse pressure was observed with no appreciable decrease in the rate.

Upon intravenous injection sigmodal sodium produced vasodilation and fall of blood pressure (Fig. 1).

These vasodepressor effects are similar to those produced by comparable doses of other barbiturates. The magnitude of the vasodilator effects depends upon the rate of injection, the size of the dose and the concentration of the solution. Slow intravenous infusion of the 0.2 per cent sigmodal sodium solution produced little or no change in blood pressure if the dose did not exceed 10 mg. Larger doses of sigmodal sodium (35 mg. or more) given by slow intravenous infusion produced a gradual fall of blood pressure terminating in circulatory collapse which occurred only after respiration had ceased for some time. Narcotic doses given intravenously or rectally for the purposes of acute laboratory experiments do not produce abnormally low blood pres-

sures. Dogs receiving 25 to 35 mg. of sigmodal sodium intravenously have an initial blood pressure of the same magnitude, 120 to 170 mm. Hg, as seen under other types of anesthesia; another dog receiving an anesthetic dose rectally had a blood pressure of 164 mm. Hg; and a rabbit receiving an anesthetic dose rectally had a blood pressure of 116 mm. Hg. Continuous administration of ether through the tracheal cannula, when the animals are under full effects of sigmodal sodium, results in a slight lowering of the blood pressure. Unless lethal or nearly lethal quantities of sigmodal are present in the animal the recoveries from the vasodepressor effects are fairly rapid.

This drug produced a slowing and decreased amplitude of the respiratory movements (Fig. 2), the slowing and decrease being proportional to the dose. With fatal doses the respiration may assume either Cheyne-Stokes rhythm or a form which is rather peculiar and perhaps characteristic of this drug. This latter type of respiration is shown in Fig. 2.

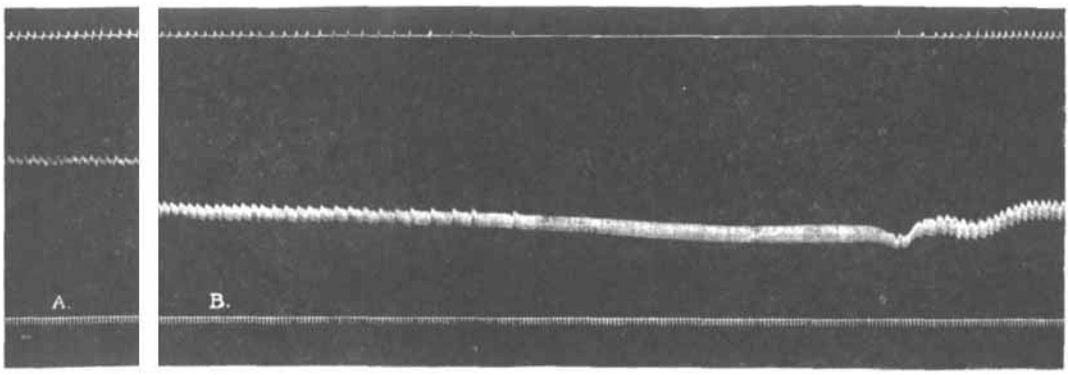

Fig. 2.—Dog, Male, Weight, 8.0 Kg., 250 mg. Sigmodal Sodium by rectum. A. One hour after administration. B. Three hours after administration. Upper line respiration, second line blood pressure, lower line base line. Time = 1.7 seconds. Animal subsequently died.

After a fairly long normal period, the respiratory rate slows gradually and apnœa occurs for a period of about three minutes during which time there is a slight fall in blood pressure. At the end of this period respiration is resumed at the previous rate and the blood pressure recovers. This phenomenon may be observed several times before death occurs from the apnoea. Death from sigmodal sodium is always a typical respiratory death.

Metrazol increases the rate and depth of respiration following respiratory depression by toxic doses of sigmodal sodium.

ANTAGONISMS

In order to study the anti-convulsant and life-saving effects of sigmodal in poisoning by convulsants, the following experiments were performed:

(*a*) Three rabbits received 30 mg. of sigmodal sodium by vein, and ten minutes later the first received 0.36, the second 0.72 and the third 1.44 mg. (per Kg.) of strychnine sulfate (*i. e.*, one, two and four average fatal doses) by vein. There were immediate convulsions in all three animals, but they recurred infrequently and, especially in the first two animals, they were not of the violent type. All three rabbits recovered, but the recovery time was prolonged in all. The last rabbit receiving four fatal doses of strychnine developed a persistent spastic paralysis of the hind limbs.

(*b*) Four hundred mg. (per Kg.) of antipyrine, administered by vein produces tonic and clonic convulsions in rabbits. In two rabbits 30 mg. of sigmodal sodium prevented the appearance of convulsions from this dose of antipyrine. The recovery time of these animals was about the same as that of the control animals receiving only 30 mg. of sigmodal sodium. The administration of antipyrine did not increase the depth of depression or analgesia in the sigmodal sodium-treated rabbits.

(*c*) Two rabbits, receiving 25 mg. each and one rabbit receiving 30 mg. of sigmodal sodium intravenously, were given eight minutes later 50 mg. of metrazol (per Kg.) by vein. Immediate return of the righting reflexes followed the injection of metrazol and final recoveries were observed twelve, thirty-four and thirty-three minutes later. (cf. Table I for normal recoveries.)

DISCUSSION

Sigmodal can apparently be extracted from the urine by methods ordinarily used for barbiturates and estimated colorimetrically. Sigmodal acid, in agreement with the results of Dille and Koppanyi (5), gives a lighter color in the cobalt test than barbital, this being due to the difference in the molecular weights. Since the cobalt color test is not specific for any particular barbiturate, there is no proof that sigmodal is excreted in the urine unchanged. If it is not changed in the

body, the relatively high percentage excretions recorded above are correct, but if it is converted to a barbiturate of lower molecular weight, the results would be correspondingly lower. Even these lower figures, however, would be sufficient to warrant a statement that sigmodal is excreted in the urine in appreciable quantities.

The margin of safety of sigmodal sodium as seen from the tables is comparable to that of other barbiturates. The margin of safety represents the ratio between the fatal and the 4+ anesthetic doses. This margin is wide enough to allow the free use of sigmodal sodium as a laboratory anesthetic. Dogs may be anesthetized safely with doses from 25 to 30 mg. by vein, and a good surgical anesthesia may be maintained for two hours or more. The operated animals show an uneventful recovery in about five hours. While sigmodal sodium may be unreservedly recommended as a laboratory anesthetic, it should not be used to produce surgical anesthesia in humans. No fixed anesthetic should be used for this purpose. Let us state definitely not only with reference to sigmodal sodium but to other barbiturates, that since small differences in potency of barbiturates cannot be detected with certainty and since animals show considerable variations in effects produced by the same dose of a barbiturate, it is impossible to calculate a safe average anesthetic dose for humans, particularly in view of the relatively narrow margin of safety of barbiturates. These considerations apply only to the use of barbiturates for complete surgical anesthesia and not to their use in pre-anesthetic medication.

Sigmodal sodium is definitely a short-acting barbiturate. As is the case with most barbiturates, it is shorter-acting in rabbits than in dogs. To study the duration of action, *i. e.*, the essential elimination of sigmodal, the method of Koppanyi and Lieberson (7) was used and also a new method which is believed to be applicable to all central depressants. This method is very simple and presupposes only the knowledge of the 50 per cent fatal dose, the average sleeping time of the survivors from this dose, and of the maximum dose which permits the maintenance of normal body posture. Since by the term "recovery" we mean the ability of the animal to maintain its normal posture unassisted at the termination of sleep, we assume that the animal is in the same condition as an animal which has received the maximum amount of the drug not interfering with its ability to maintain normal body posture. Therefore, the formula —50 per cent fatal dose minus maximum dose permitting normal body posture, divided by the sleeping time of the survivors—discloses the amount of drug essentially eliminated per unit of time. The results obtained by the two different methods agree fairly closely (18 mg. *vs.* 15 mg. per hour).

Determination of the essential elimination of a hypnotic by the intravenous method should enable one to predict the average sleeping time by the oral, rectal or other routes of administration when anesthetic doses are given, provided there is complete absorption of the drug. The results from the second method show that 15 mg. of sigmodal sodium are essentially eliminated during one hour. By modifying the formula to calculate for sleeping time instead of elimination, it may be estimated that rabbits receiving 90 mg. of sigmodal sodium rectally should recover in 320 minutes $\left(\frac{90 - 10}{15} = 5 \text{ hrs. } 20 \text{ min.}\right)$; rabbits receiving 80 mg. should recover in 280 minutes, and rabbits receiving 60 mg. should recover in 200 minutes. Considering the relatively small number of animals used for the determination of sleeping time in Table II, the agreement between the theoretical and the actual sleeping times is remarkably close. According to Boedecker and Ludwig (8), rabbits receiving 400 mg. of sigmodal orally recover from their narcotic sleep in 24 hours or more. By the above method of calculation $\left(\frac{400 - 10}{15} = 26 \text{ hours}\right)$, the theoretical time of recovery again closely approximates the actual experimental results. From protocol data available in the laboratory on 3,3-diethyl-2,4-dioxotetrahydropyridine predictions were made on the sleeping time from various doses; these checked well with the experimental observations. We feel, there-

fore, that this method of estimating sleeping times may be applied to other central depressants.

The narcosis produced by sigmodal is similar to that produced by other short-acting barbiturates. It should be emphasized, however, that in rabbits the onset of action by rectal administration is very rapid and fairly constant. This agrees with the results of Boedecker and Ludwig (8) and Reichold (9).

The effects of this drug on the heart, circulation and respiration are not different from those of other barbiturates. We mentioned the fact that fatal doses may produce a rather unique type of Cheyne-Stokes respiration where the periods of respiratory movements and of the apnœa are very prolonged.

Sigmodal sodium suppresses convulsions from antipyrine and strychnine and saves the lives of animals from four fatal doses of strychnine given by a single injection. This indicates a rather powerful anti-convulsant action. Antipyrine, which is added by the manufacturer to the standard rectal solution of sigmodal sodium, does not appear to increase its toxicity or its analgesic effect.

Metrazol was found to be an efficient analeptic in sigmodal sodium narcosis. It could be and already has been (10), (11), (12), (13) used with benefit in sigmodal poisoning and in conditions in which the immediate interruption of sleep appeared to be desirable.

SUMMARY

1. The 50 per cent fatal dose of sigmodal sodium for rabbits is 40 mg. (intravenous) and 90 mg. (rectal), and for dogs, about 35 mg. (intravenous).

2. Sigmodal sodium is a depressant of the central nervous system producing in appropriate doses in rabbits loss of righting reflexes, motor paralysis, muscular relaxation and a deep sleep from which the animals cannot be aroused. In the dog it produces true surgical anesthesia.

3. The onset of action of sigmodal sodium after intravenous injection is immediate in all animals and after rectal administration it occurs within a few minutes in rabbits.

4. The rate of essential elimination of sigmodal sodium was found to be 37 to 47 per cent of the average fatal dose (15 to 19 mg.) per hour as determined by two different methods, one of which is described herein for the first time.

A method for estimating sleeping time after rectal, oral and other routes of administration applying the data derived from the intravenous essential elimination determination is described.

5. The drug given intravenously is a circulatory and respiratory depressant comparable to other short-acting barbiturates. The blood pressure and respiratory rate in animals anesthetized with sigmodal sodium are similar to those obtained under other anesthetic agents.

6. Sigmodal sodium, even in fatal doses, does not abolish the cardiac slowing from faradic stimulation of the vagus. The cardiac and vasomotor responses to autonomic drugs are unchanged.

7. Sigmodal sodium has definite anticonvulsant properties and conversely, a central stimulant such as metrazol antagonizes its depressant effects.

8. Large doses of antipyrine do not deepen sigmodal sodium narcosis or alter the recovery time.

9. Sigmodal sodium may be estimated in body fluids by the cobalt-color tests. About one-fifth of the dose of the drug (or its end products) is excreted in the urine within 48 hours following administration.

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Further Observations on the Influence of the Anesthetic on the Results of Digitalis Assay by the Cat Method of Hatcher and Brody

By Chas. C. Haskell

In 1936, data were presented, which established that the cat unit for certain preparations of digitalis was materially higher when a non-volatile anesthetic, dial-urethane solution, was substituted for ether in the Hatcher-Brody method (9). The only earlier published reports regarding the possible influence of a non-volatile anesthetic on the size of the cat unit encountered were in the papers of Epstein (4), David and Rajaminickam (2), and Bauer and Fromherz (1), but, with the possible exception of the last-mentioned, the number of experiments performed by these authors seems scarcely to justify the conclusions drawn. This criticism is not applicable to the later publication by Edmunds, Moyer and Shaw (3), in which it was pointed out that, with animals under urethane anesthesia, the cat unit for the International Powder was considerably higher than was the case when etherized animals were used. The practical importance of departing from the original technique of the Hatcher-Brody method by substitution of a non-volatile anesthetic is immediately obvious; in addition, the interesting question arises why etherized cats succumb to smaller doses of digitalis than do those under the influence of dial-urethane or urethane. Does ether in some way directly lower the resistance of the cats to digitalis poisoning or, on the other hand, do the non-volatile anesthetics mentioned oppose, in some specific manner, the lethal action of this drug? Another possibility, suggested by Dr. Harry Gold (5), is that the influence of the non-volatile anesthetic is indirect; by prevention of struggling, it delays the onset of ventricular fibrillation, so prone to be precipitated by a struggle in unanesthetized cats after large doses of digitalis. If it is found that other non-volatile anesthetics, chemically unrelated to dial or urethane, affect the resistance of cats to digitalis as do these latter, it would tend to support, but not establish the correctness of Dr. Gold's explanation.

David and Rajaminickam (2) assayed a specimen of the International Powder on five series of cats, using ether, urethane, chlorobutanol, chloralose or paraldehyde in the different series. They report that the chlorbutanol series gave the largest cat unit; the smallest being obtained from the chloralose series; while that from the etherized cats was intermediate in size. Such results would seem to indicate that the various anesthetic agents acted in a specific manner to affect the resistance of the cats to digitalis intoxication, but when it is taken into consideration that the number of animals in each series was only five, except in case of paraldehyde, where four were used, it is obvious to anyone familiar with the practical use of this method that the differences recorded are without significance. It is of interest to note that David and Rajaminickam found the ether cat unit larger than when urethane was used, in contrast to the results obtained by Edmunds, Moyer and Shaw (3) from a much larger number of animals. As already stated (8) a limited number of experiments in our laboratory in which the cat unit for animals anesthetized with chlorbutanol was compared with that obtained with etherized cats, failed to show any material difference, but here, too, the number of experiments was far too small to justify positive conclusions. It seemed desirable, therefore, to make further observations, in the attempt to determine whether the substitution of chlorobutanol for ether anesthesia affects the size of the cat unit.

EXPERIMENTAL

In the first experiments, the procedure was similar to that employed in comparing results under dial-urethane and ether anesthesia. Groups of ten cats were used for each assay; five of the animals received 200 mg. chlorbutanol per Kg. intraperitoneally, a 40% solution in ethylene glycol being used; the other five being lightly etherized. The