SECTIONS

AN INVESTIGATION OF ACQUIRED TOLERANCE TO CERTAIN SHORT-ACTING BARBITURATES.*

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The existence of tolerance to the short-acting barbiturates has been a matter of disagreement in the literature. Fitch (1) studying the effect of daily administrations of neonal, noctal and amytal by stomach tube in rabbits reported "a high degree of tolerance" for all of these drugs as indicated by the greater lethal dose in the treated animals. Ravdin, Drabkin and Bothe (2) administered 50 to 150 mg. of amytal to rats for histological studies and reported that there was no tolerance in that sleep was always produced in the same time. Deuel, Chambers and Milhorat (3) state that they found no tolerance in dogs when amytal was given at intervals of several days. Nicholas and Barron (4) report that rats "which have had one or more injections of sodium amytal occasionally develop a very high resistance to the drug." Swanson, Weaver and Chen (5) made extensive studies on the tolerance to amytal in ten dogs and six monkeys. The sodium amytal was given three times a week for periods varying from two to six months. They found no change in the lethal dose for the amytalized dogs. In both the dogs and monkeys the period of sleep was slightly less after the administration had been continued than it was at first.

Using pentobarbital-sodium Swanson and Shonle (6) administered anesthetic doses intravenously to three dogs at 48-hour intervals for 8 days. They found a decrease in the sleeping time of two dogs and an increase in the third. The differences were ascribed to individual variations in response. Stanton (7) using rats found only a very minor degree of tolerance developed after daily administration of nembutal as indicated by the response of the animals to a uniformly uncomfortable situation. On the other hand Moir (8) found tolerance "usually at the second injection." Carmichael and Posey (9) also using pentobarbital found that the length of hypnosis was shortened by the daily administration of 25 per cent of the Minimal Fatal Dose. This was also shown with semi-weekly administration. Data on the other short-acting barbiturates is meager. Kennedy (10) reports that evipal "does not appear to be cumulative or to produce tolerance." With regard to clinical experience, the assumption has been made (11, 12, 13, 14) that there is a low degree of tolerance developed in humans but this is not supported by conclusive evidence.

Part of the discrepancy in the work reported in the literature is due to the fact that the study of tolerance was incidental to some other work or that different experimental procedures were used in different laboratories. Assuming acquired

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tolerance to be—a condition developed by the frequent administration of a drug in which the original response to the drug decreases—we proceeded to investigate the development of a decreased response to five of the common short-acting barbiturates: pentobarbital, pernoston, amytal, ortal and evipal.[1]

EXPERIMENTAL METHODS AND RESULTS.

The Production of Acquired Tolerance.—Adult male albino rabbits were used in these experiments. They were fed Alber's Rabbit Pellets with occasional greenstuff. The general plan involved the daily intravenous administration of a definite anesthetic dose of the barbiturate with a careful measurement of the resulting period of sleep. The sleeping time was taken as the period during which the righting reflex was absent. This reflex disappeared almost immediately after the injection of the drug and the time of recovery was determined by placing the rabbit on its back and noting the time when there was a spontaneous successful effort to right itself. The reflex was tested at two-minute intervals and was usually sufficiently definite to assure an accurate estimate of the period of sleep.

The doses used were as follows: Pentobarbital-sodium, 25 mg. per Kg.; Pernoston, 40 mg. per Kg.; Amytal-sodium, 35 mg. and 40 mg. per Kg.; Ortal-sodium, 45 mg. per Kg.; and Evipal-sodium, 40, 60 and 80 mg. per Kg. The doses were calculated daily on the basis of the body weight. Fresh solutions were always used and the injection was always made at the same rate.

The results of the daily intravenous administration of these barbiturates are tabulated in Table I and summarized in Fig. 1. The average sleeping time shows a distinct decrease in the

TABLE I.—THE PRODUCTION OF TOLERANCE TO CERTAIN SHORT-ACTING BARBITURATES.

No. of Rabbits.	Barbiturate.	Dose, Mg./Kg.	Day of Administration.	Average Sleeping Time.	Standard Deviation.
			1	36.4	6.0
			2	32.2	4.9
			3	24.8	2.9
			4	22.4	3.4
			5	24.2	5.2
5	Pentobarbital	25	6	24.0	4.3
			7	22.4	2.9
			8	20.4	2.1
			9	23.8	1.6
			10	25.4	2.1
			11	24.4	3.2
			1	69.0	11.8
			2	45.4	12.5
			3	44.0	7.1
			4	40.8	8.4
5	Pernoston	40	5	43.4	8.1
(3 after the 8th			6	42.6	9.4
injection)			7	41.0	10.0
			8	41.0	10.3
			9	40.0	8.6
			10	39.6	9.0
			11	40.0	9.3

[1] Pentobarbital, ethyl (1 methyl butyl) barbituric acid; Pernoston, butyl bromallyl barbituric acid; Amytal, isoamyl ethyl barbituric acid; Ortal, *n*-hexyl ethyl barbituric acid; Evipal, N-methyl cyclohexenyl methyl barbituric acid. For convenience the common names of these drugs are used in the text. All except pernoston were used in the form of the sodium salt and the doses, except for pernoston, are stated as the sodium salt.

We wish to acknowledge the generosity of the following companies for supplying us with the barbiturates used in this work: Pentobarbital (Nembutal), Abbott Laboratories; Pernoston, Riedel-de Haen, Inc.; Amytal, Eli Lilly and Company; Ortal, Parke, Davis and Company; Evipal, Winthrop Chemical Company.

TABLE I.—(*Continued from page 831.*)

			1	46.2	19.8
			2	27.3	7.5
			3	27.0	5.4
			4	25.1	3.2
5	Amytal	35	5	28.4	4.4
			6	26.2	4.6
			7	30.0	5.8
			8	27.1	7.2
			9	31.3	12.9
			10	28.2	10.7
			1	85.6	18.9
			2	54.2	7.3
			3	46.0	7.4
			4	38.1	2.4
3	Amytal	40	5	32.0	1.9
			6	28.2	2.6
			7	28.0	0.3
			8	26.4	1.9
			9	26.3	1.4
			10	25.2	2.5
			1	23.2	6.2
			2	26.4	6.5
5	Ortal	45	3	26.6	7.7
(3 after the 6th injection)			4	26.2	6.1
			5	29.2	7.4
(2 after the 8th injection)			6	29.4	3.2
			7	34.0	2.8
			8	31.0	8.8
			9	29.5	8.5
			1	17.6	1.4
			2	16.0	1.7
			3	16.6	1.8
			4	17.6	1.5
			5	17.0	1.4
			6	17.4	1.3
			7	19.0	1.5
5	Evipal	40	8	17.4	1.8
			9	17.6	1.9
			10	17.4	1.8
			11	18.0	1.8
			12	17.8	1.6
			13	17.6	1.1
			14	17.8	2.0
			15	17.4	2.3
			1	34	...
			2	33	...
			3	28	...
			4	32	...
1	Evipal	60	5	32	...
			6	26	...
			7	26	...
			8	28	...
			9	29	...

			1	41	...
			2	42	...
			3	28	...
			4	32	...
1	Evipal	80	5	32	...
			6	26	...
			7	26	...
			8	28	...
			9	29	...

case of pentobarbital and pernoston, both in the individual animals and in the averages. The averages for amytal show a decrease but in two animals one did not show a decrease and one

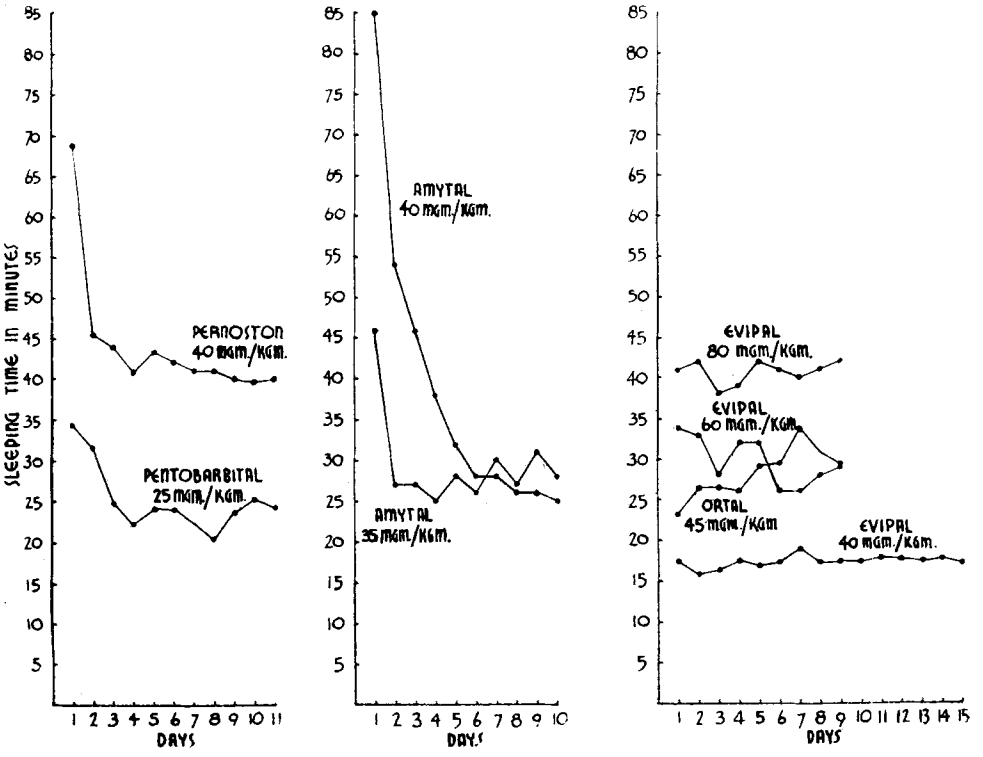

Fig. 1.—The average sleeping time produced by five short-acting barbiturates upon daily administration. The graphs for pernoston, pentobarbital, amytal (35 mg. per Kg.), evipal and ortal are averages of the sleeping time of 5 rabbits. Amytal (40 mg. per Kg.) is the average sleeping time of 3 rabbits and evipal (80 and 60 mg. per Kg.) is the sleeping time of one rabbit. The daily administration was continued with one rabbit in the pentobarbital, pernoston and amytal (35 mg. per Kg.) series for a total of 30 days without further significant change in the sleeping time.

showed a greater response. There was no significant decrease in the time of sleep produced by ortal or evipal. The daily administration was continued for 30 days for one rabbit from each of the pentobarbital, pernoston and amytal series. After the preliminary decrease in sleeping time which leveled off in about 5 days as shown in the graph there was no further significant change. The average sleeping time after the development of tolerance for pentobarbital was approximately 70 per cent of that produced by the first dose, for pernoston 73 per cent, and for amytal 65 per cent. There was considerable variation between animals as will be seen from the standard deviations in Table I.

These results lead to the assumption that in rabbits acquired tolerance can be easily produced by the daily administration of pentobarbital and pernoston, less easily by amytal and not at all by ortal and evipal.

The Duration of the Acquired Tolerance.—After tolerance had developed as shown by a marked decrease in the sleeping time the daily administration was stopped for various periods of time and the drug again administered and the sleeping time determined. If this was approximately the same as that produced by the first dose of the drug the assumption was made that the acquired tolerance had been lost. Table II shows the results of experiments of this sort

TABLE II.—DURATION OF ACQUIRED TOLERANCE TO PENTOBARBITAL, PERNOSTON AND AMYTAL.

Rabbit No.	Barbiturate.	Dose, Mg./Kg.	Original Sleeping Time, Min.	Days to Produce Tolerance.	Sleeping Time before Period of Non-Administration. Per Cent of Original Sleeping Time.	Days of Non-Administration.	Sleeping Time after Period of Non-Administration. Per Cent of Original Sleeping Time.
1			45	11	44	2	46
5			31	7	80	2	90
2			42	11	54	3	69
5			33	6	72	3	93
5	Pentobarbital	25	34	11	79	4	97
2			29	5	75	4	100
4			31	11	74	5	87
2			29	8	82	5	96
3			33	11	87	6	125
4			32	7	73	6	115
9			55	12	49	2	56
8			65	10	86	2	89
7			55	9	80	3	90
6			55	6	78	3	90
8	Pernoston	40	89	8	56	4	73
7			73	7	78	4	87
6			63	12	65	5	88
6			51	6	84	6	117
7			64	7	87	6	104
13			59	11	30	2	37
15			47	7	68	3	68
14			58	6	72	3	62
12	Amytal	35	64	11	35	3	30
14			65	11	64	4	81
14			53	7	70	5	109
15			33	4	82	6	112

for pentobarbital, pernoston and amytal. The sleeping time produced by the first dose is taken as 100 per cent and the sleeping time after the production of tolerance is expressed as a part of this percentage. After the interval of non-administration the sleeping time was again determined and is expressed in Table II as percentage of the original sleeping time. It can be seen that in the case of pentobarbital, tolerance is lost quite rapidly and the response to this drug is approximately equivalent to the original at the end of 3 days. With pernoston and amytal the tolerance is lost in 4 or 5 days.

The Rate of Elimination of Amytal in Tolerant and Non-Tolerant Animals.—The short-acting barbiturates are generally assumed to be destroyed almost completely in the body and there is, therefore, the possibility that tolerance can be explained on the basis of a more rapid destruction of these hypnotics. To investigate this, the rate of disappearance of a dose of 40 mg. per Kg. of sodium amytal injected intravenously was determined in the blood, liver and muscle

tissue of normal rabbits and rabbits which were made tolerant to amytal. Four series of 4 rabbits each were used. One series was the control, and the rate of disappearance of amytal was tested without any previous medication. The other three series were given 35 mg. of sodium amytal per Kg. daily for 2, 4 and 6 weeks, respectively, and the rate of the disappearance determined at the end of these periods. One day elapsed between the end of the daily injections and the determinations.

Amytal was determined by the colorimetric test of Koppanyi, *et al.* (15, 16). The animals were injected with the drug and at the end of the proper time interval killed by bleeding from the carotid arteries. The total blood from one animal was used for one determination. Liver and muscle tissue was taken at the same time for analysis.

These findings are summarized in Fig. 2 which shows that the amytal disappears at a slightly greater rate in the tolerant animals. The difference is greatest in the case of blood. Here, there is the added factor of the absorption of the drug by the tissues and this may mean that the tissues of tolerant animals also take up the drug from the blood stream at a more rapid rate.

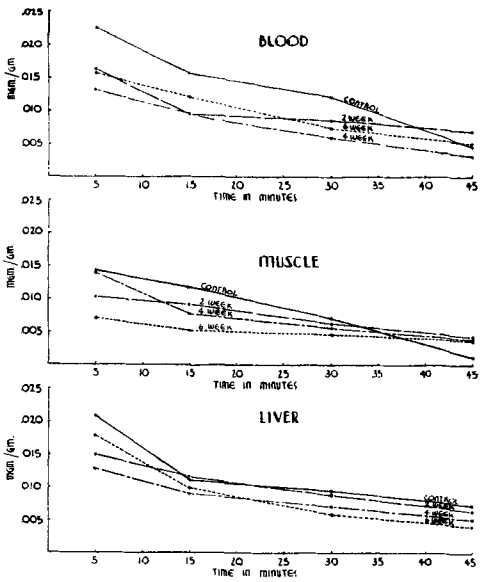

Fig. 2.—The rate of disappearance of a dose of 40 mg. per Kg. of amytal from blood, liver and muscle in tolerant and non-tolerant rabbits. Tolerance was developed by the daily subcutaneous administration of 35 mg. of amytal per Kg. for 2, 4 and 6 weeks.

DISCUSSION AND SUMMARY.

The definite decrease in sleeping time which resulted from the daily administration of pentobarbital and pernoston and at least partially for amytal, seems to us evidence that a tolerance may be acquired for these barbiturates. In view of the fact that there was no significant change in the sleeping time during a period of daily administration or ortal and evipal it does not seem probable that the decrease in sleeping time is simply day-to-day variations in the response of the animals. The tolerance to pentobarbital, pernoston and amytal is of a comparatively low degree, since after it has developed the sleeping time is only approximately 70 per cent of that produced by the first dose. Furthermore, after a few days, a basal response is reached which does not significantly change even though the administration is carried on for as long as a month. This might be of clinical significance if these drugs are used for anesthetic purposes in patients who have received them previously. In pharmacological experiments involving the production of anesthesia in animals on successive days we have observed this decreased response and have had to administer larger doses to produce the same degree of anesthesia. Tolerance is lost quite rapidly and within 3 or 4 days after the daily administration has been stopped, the animals respond in approximately the same way as they did to the original dose.

A possible explanation for the tolerance may lie in an increased ability of the organism to destroy the barbiturate. Our experiments point to this but are not

conclusive. Further experiments are being carried out to test this possibility further.

CONCLUSIONS.

Using sleeping time as the criterion, acquired tolerance to amytal, pentobarbital, ortal, pernoston and evipal was investigated in rabbits. It was found that upon daily administration of ortal and evipal there was no significant change in the sleeping time. Tolerance to a certain degree was developed for pentobarbital, pernoston and amytal as evidenced by a significant decrease in sleep. The tolerance developed almost immediately following the first injection, and reached its limits in four to seven days.

It was found that the acquired tolerance disappeared rapidly after the ending of the daily injections and within 3 or 4 days the animal responded to the barbiturate in practically the same way as it had done to the first dose.

The destruction of amytal as determined by its rate of disappearance from the blood, liver and muscle appears to take place somewhat more rapidly in tolerant than in non-tolerant rabbits.

BIBLIOGRAPHY.

(1) Fitch, R. H., *J. Pharmacol.*, 39, 266 (1930).
(2) Ravdin, I. S., Drabkin, D. L., and Bothe, A. E., *J. Lab. Clin. Med.*, 16, 561 (1930).
(3) Deuel, H. V., Chambers, W. H., and Milhorat, A. T., *J. Biol. Chem.*, 69, 249 (1926).
(4) Nicholas, J. S., and Barron, D. H., *J. Pharmacol.*, 46, 125 (1932).
(5) Swanson, E. E., Weaver, M. M., and Chen, K. K., *Am. J. Med. Sci.*, 193, 246 (1937).
(6) Swanson, E. E., and Shonle, H. A., *J. Lab. Clin. Med.*, 16, 1056 (1931).
(7) Stanton, E. J., *J. Pharmacol.*, 57, 245 (1931).
(8) Moir, W. M., *Ibid.*, 59, 68 (1937).
(9) Carmichael, E. B., and Posey, L. C., *Proc. Soc. Exptl. Biol. Med.*, 30, 1329 (1933).
(10) Kennedy, W. P., *J. Pharmacol.*, 50, 347 (1934).
(11) Wagner, C. P., *J. Am. Med. Assoc.*, 101, 1787 (1933).
(12) Hoge, S. F., *Am. Med.*, 40, 235 (1934).
(13) *J. Am. Med. Assoc.*, 105, 1374 (1935).
(14) Schmitz, H. E., *Am. J. Obst. Gynecol.*, 33, 103 (1937).
(15) Koppanyi, T., Murphy, W. S., and Krop, S., *Arch. intern. pharmacodynamie*, 46, 76 (1933).
(16) Koppanyi, T., Dille, J. M., Murphy, W. S., and Krop, S., JOUR. A. PH. A., 23, 1074 (1934).

THE DETERGENT QUALITIES OF SOFT SOAPS.*

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This work was undertaken to determine the relative efficiency of soaps made from the common fixed oils and Oleic Acid which were thought best suited for a soft soap. The soft soap of the Pharmacopœia is very effective but somewhat objectionable because of its odor and color and is not generally used. Soaps made with Sodium and Potassium Hydroxides in the ratio indicated in the U. S. P. XI

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