14451

Duration of Sleep Produced by Pentobarbital Sodium in Normal and Castrate Female Rats.*

CHARLES GAYLORD AND HAROLD C. HODGE.

From the Department of Biochemistry and Pharmacology, The School of Medicine and Dentistry, The University of Rochester, Rochester, New York.

Cameron[1] reported an "unexpected result" —that following a given dose of pentobarbital sodium (nembutal), castrate female rats slept twice as long as did normal female rats. Specifically, he found the mean duration of sleep to be 223 ± 12 minutes for the castrated animals, and 100 ± 4 minutes for the normal rats following 40 mg of pentobarbital per kilo of body weight. He attributed the longer sleep of the castrated rats to the established tendency of castration to depress metabolism.[2,3] The depression in metabolism, he thought, would decrease detoxification and increase the susceptibility to barbiturates. Barbiturates in certain doses are known to depress oxygen consumption and body temperature.[4,5,6] Consequently in the presence of a depressed metabolic state, it is logical to assume that the duration of the action would be lengthened.

The difference of average duration of sleep between normal and castrate rats was so striking that we believed the experiment worthy of repetition. To our surprise we found that the normal animals slept about twice as long as did the castrate rats (Table I); and, in fact, our data are numerically quite similar to those of Cameron but the effect is reversed. Thus the normal rats slept 261 ± 69 minutes and the castrate rats slept 118 ± 59 minutes (Test 2) following the subcutaneous administration of 30 mg† of pentobarbital sodium per kilo of body weight.

The data in Table I are in line with the findings of Holck *et al.*[7] who have shown that spayed female rats develop a tolerance to pentobarbital more readily than do normal female rats. Holck *et al.* used another method for observing the effect of castration and his rats had been spayed for a somewhat shorter period. They observed that larger doses of pentobarbital are required to produce a given effect in spayed female rats; our data show a greater effect (duration of sleep) in normal female rats following a stated dose.

We are at a loss to explain the contradiction between our data and those of Cameron. However, on repeated tests at room temperature (Table I-B), at lower temperatures (Table I-A), and at higher temperatures (Table I-C), the normal rats uniformly slept significantly longer than did the castrate rats in each case. We are inclined to attribute the shorter duration of sleep of the castrate rats to their extra subcutaneous fat. Cameron showed that warmth tended to decrease the duration of sleep following pentobarbital The insulating effect of the subcutaneous fat would tend to maintain a higher body temperature in castrate rats. It may be seen from Table I that castrate rats averaged about 290 g body weight while the normal rats averaged about 240 g; these were significant differences in every case. The castrate rats had been operated at the age of one month and were not

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1 Cameron, G. R., *Proc. Roy. Soc. Med.*, 1938, **32**, 309.

2 Loewy, A., and Richter, P. F., *Arch. Anal. Physiol.*, 1899, **174**, supp. 175.

3 Loewy, A., *Zentralblatt Physiol.*, 1902, **16**, 449.

4 Anderson, H. H., Chen, M. Y., and Leake, C. P., *J. Pharm. Exp. Therap.*, 1930, **40**, 215.

5 Dameshek, W., Myerson, A., and Loman, J., *Am. J. Psychiat.*, 1934, **91**, 113.

6 Shapiro, L. B., *J. Nerv. Ment. Dis.*, 1937, **85**, 305.

† We found that 40 mg per kilo body weight—the dose used by Cameron—resulted in unfavorably high mortalities.

7 Holck, H. G. O., Mathieson, D. R., Smith, E. L., and Fink, L. D., *J. Am. Pharm. Assn.*, 1942, **31**, 116.

TABLE I.
Data on Duration of Sleep of Rats Given 30 mg of Pentobarbital per kg Body Weight.

Test No.		No. rats	Avg body wt, g	Avg induction time, min.	Avg time asleep, min.	No. asleep	Probability of significant difference: N-C (P)	No. rats died
			Part A: Air Temperature—13° C.					
4	Normal	18	243	31	247 ± 105	16		1
							.15-.10	
	Castrate	17	292	36	168 ± 113	10		1
6	Normal	17	248	8	292 ± 118	16		0
							<.01	
	Castrate	17	295	29	161 ± 95	13		0
			Part B: Air Temperature—23°C.					
2	Normal	20	243	23	261 ± 69	20		1
							<.01	
	Castrate	19	297	45	118 ± 59	16		1
7	Normal	16	244	36	276 ± 90	16		1
							<.01	
	Castrate	17	293	62	166 ± 70	12		0
			Part C: Air Temperature—37°C.					
3	Normal	17	243	31	173 ± 42	16		1
							<.01	
	Castrate	18	296	39	109 ± 84	12		1
5	Normal	19	236*		186 ± 78	19		0
							.02-.01	
	Castrate	19	287		88 ± 52	15		0

* Avg wt of 17 rats.

tested experimentally until 12 months later. This is the procedure‡ that Cameron had followed, and he reported that his castrate rats weighed 240 to 355 g whereas his normal rats weighed from 160 to 350 g.

The average induction time was greater in each instance for the castrate rats than for the normal rats (Table I). The induction time was taken as the interval between the time of injection subcutaneously of the pentobarbital solution along the lateral portion of the abdominal skin and the time when the rats could be lifted carefully from the cage and laid, still asleep, on the cage top. The duration of sleep was taken as the interval between the time of this transfer and the time when the rat had sufficiently awakened to move itself off the cage top. Both the induction time and the "time asleep" were quite variable. The induction time for the normal rats averaged about 30 minutes in most tests, but was as high as 45 to 62 minutes (Tests 2 and 7) for the castrate rats. Only rarely did all of the rats go to sleep (Table I); the average "time asleep" is that of the rats which slept. In each test the normal rats' duration of sleep was significantly greater than that of the castrate rats; the probabilities calculated according to Fisher's "t" test[8] are given in Table I.

The mortality did not differ significantly between the normal and castrate groups, 4 of the normal rats died during the various experiments and 3 of the castrates died. This also is not in accord with Cameron's finding that 22% of the castrate rats died and none of the normal rats. However Holck *et al.*[7] have shown that 2 to 3 times as much pentobarbital is required to kill the average spayed female rat as to kill the normal female control

‡ Cameron used rats aged 12 months which had been spayed at age 1 month. Thus, Cameron's rats were 1 month younger than ours; this difference in age is probably negligible.

[8] Fisher, R. A., *Statistical Methods for Research Workers*, London, 7th edition, 1938, 128.

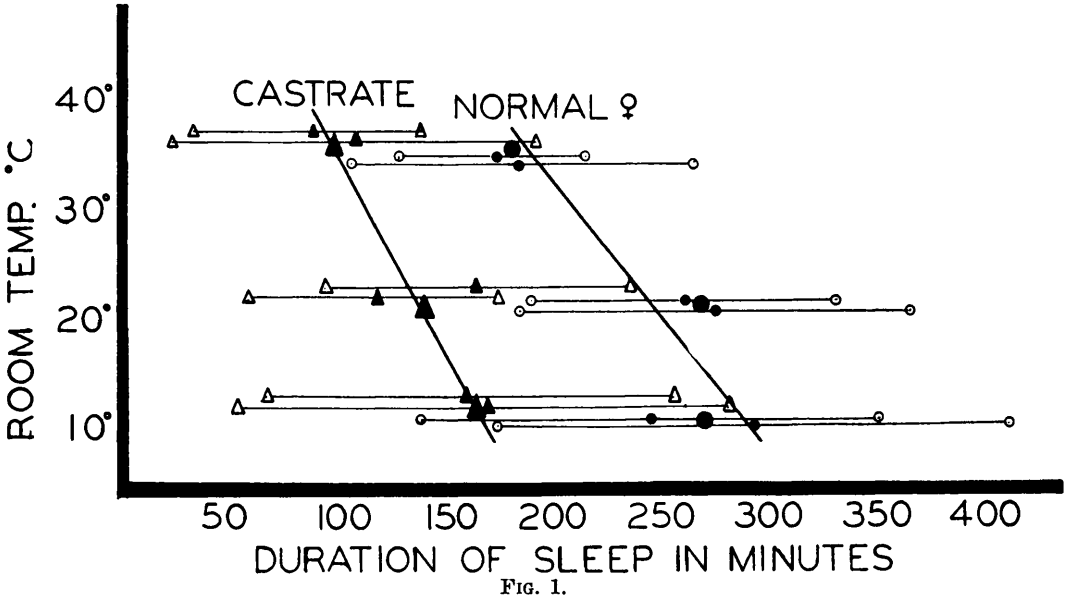

FIG. 1.

Duration of sleep at various room temperatures. Although wide variations in the average durations of sleep in both normal and castrate female rats are observed at each room temperature, there are consistent tendencies toward shorter sleep periods with increases in room temperature.

rat. Our results are in accord with Holck's; castration increases the resistance of the female rat to pentobarbital.

Both the normal and castrate rats showed a tendency to a shorter duration of sleep at increasing temperatures (Fig. 1). The differences between the durations of sleep at the lower temperature (13°C) and at room temperature (23°C) were of doubtful significance for both the normal and castrate groups. However, significant differences were obtained in each case between the durations of sleep at room temperature and at the higher environmental temperature (37°C). This finding is in agreement with the observations of Cameron and also with the clinical observation that barbiturate poisoning may be treated by fever.

Summary. Normal and castrate female rats were given 30 mg of pentobarbital per kilo of body weight subcutaneously. The normal rats slept significantly longer than did the castrate rats. Both groups of rats were exposed to various environmental temperatures, specifically 13°, 23°, and 37°C. In general, the duration of sleep decreased with the increase of environmental temperature.