Latent Hypersensitivity to Pentobarbital in the Rat.* (30846)

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Moir(1) reported that female rats made tolerant to pentobarbital exhibited an increased susceptibility to the depressant effects of the drug 3 to 5 weeks after withdrawal of the agent. A similar phenomenon was incidentally observed by Aston(2) in male rats using a 10- to 12-day abstinence period following the induction of acute tolerance to pentobarbital. In neither of these reports was there adequate control of experimental variables such as body weight. The present study was, therefore, undertaken to reaffirm, under adequately controlled conditions, the development of delayed hypersensitivity to pentobarbital in both male and female rats. In view of the well-documented role of hepatic microsomal enzyme systems in the production of tolerance to barbiturates(3,4), it seemed to be of further interest to obtain an indication of what role, if any, might be played by liver in the genesis of post-tolerance hypersensitivity. Changes in hepatic oxidative enzyme activity were assessed by estimation of the rate of disappearance of pentobarbital from the blood of treated animals.

Methods. In the determination of post-tolerance hypersensitivity to pentobarbital in the male rat, 4 groups of animals of the Holtzman strain were employed. The animals were treated on days 1, 2, 29, and 30 according to the scheme outlined in Table I with either 40 mg/kg sodium pentobarbital, administered intraperitoneally (i.p.), or with 2 ml/kg of 0.9% aqueous sodium chloride i.p., and sleeping times recorded. Sodium pentobarbital was administered as a 1% aqueous solution made isotonic by addition of sodium chloride. A similar scheme was used for assessment of post-tolerance hypersensitivity to pentobarbital in female rats except that a dose of 30 mg/kg sodium pentobarbital i.p. was employed.

Sleeping times were measured as the time elapsing between loss of righting reflex and the point at which the anesthetized animals righted themselves and made spontaneous locomotor efforts. The sleeping time of any rat, which exhibited either an induction or sleeping time greater than the mean time of its experimental group $\pm$ 4 standard deviations, was omitted from the recorded data. Any animal whose body weight on day 30 fell outside of the mean body weight for its group $\pm$ 10% was similarly excluded from consideration in this study.

In these experiments, livers were excised on day 30 under ether anesthesia, after the rats had recovered from the anesthetic effects of their final dose of barbiturate. The livers were washed in cold, isotonic potassium chloride, blotted dry and weighed. Liver weights were expressed as grams wet liver per 100 g body weight.

The rate of decline of pentobarbital blood levels in pre-tolerant, tolerant and post-tolerant female rats was determined as an indirect estimate of the rate of metabolism of the drug. Three groups of adult female rats, weighing from 150 to 250 g, were studied. The pre-tolerant group consisted of untreated rats. The tolerant group were rats which had received 30 mg/kg sodium pentobarbital i.p. 24 hours prior to testing. The post-tolerant group were animals which received 2 consecutive doses of 30 mg/kg of the barbiturate i.p. 28 and 29 days, respectively, prior to testing. Each of the 3 groups of animals was challenged on the test day with 30 mg/kg sodium pentobarbital and blood samples were drawn by cardiac puncture, from members of each group, at 15, 30, 60, 120 and 180 minutes following administration of the barbiturate. Each rat received 1.0 ml heparin (10

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TABLE I. Mean Sleeping Time (min) ± S.E. of Male Rats Given 40 mg/kg Sodium Pentobarbital i.p.

Group	Day 1	Day 2	Day 29	Day 30
Control	Saline	Saline	Saline	Saline
Pre-tolerant	Saline	Saline	Saline	77.0 ± 3.4 (18)
Tolerant	Saline	Saline	71.5 ± 3.0 (24)	57.0 ± 2.4 (22)
Post-tolerant	103.5 ± 7.2 (19)*	62.0 ± 3.4 (21)	Saline	85.5 ± 2.6 (24)

* No. of animals.

TABLE II. Mean Sleeping Time (min) ± S.E. of Female Rats Given 30 mg/kg Sodium Pentobarbital i.p.

Group	Day 1	Day 2	Day 29	Day 30
Control	Saline	Saline	Saline	Saline
Pre-tolerant	"	"	"	187.0 ± 9.2 (14)
Tolerant	"	"	197.5 ± 14.4 (12)	88.0 ± 4.7 (17)
Post-tolerant	197.5 ± 7.9 (30)*	95.0 ± 3.8 (29)	Saline	213.5 ± 5.3 (26)

* No. of animals.

mg/ml) i.p. 15 minutes before cardiac puncture. Plasma levels of sodium pentobarbital were determined spectrophotometrically at 260 mμ according to the method of Williams and Zak(5), using a Beckman Model DB spectrophotometer. The results were expressed as μg barbiturate per ml whole blood, assuming a hematocrit of 40 cc packed red cells per 100 cc whole blood in the rat.

Statistical procedures employed were those outlined by Burn *et al*(6). All error estimates given in this report refer to standard error of the mean (S.E.), unless otherwise indicated. The significance of differences between means was, in all cases, established by application of the 'Student' t-test.

Results. Hypersensitivity to pentobarbital in the male rat. Table I lists the results of the study of latent hypersensitivity to pentobarbital in the male rat. The body weights of these animals ranged, on day 1, from 154 to 208 g, and on day 30, from 269 to 330 g (mean = 299 g). The mean sleeping times for the pre-tolerant, tolerant and post-tolerant groups on day 30 were 77.0 ± 3.4, 57.0 ± 2.4 and 85.5 ± 2.6 minutes. This was a significant decrease in sleeping time in the tolerant compared to the pre-tolerant group ($P = 0.01$). Sleeping time in the post-tolerant group was increased 11.0% over the pre-tolerant group. This increase, however, was not statistically significant ($P = 0.05$).

Hypersensitivity to pentobarbital in the female rat. The results of the study of post-tolerance responsiveness to pentobarbital in the female rat are given in Table II. The body weights of these animals ranged, on day 1, from 137 to 167 g, and on day 30, from 179 to 219 g (mean = 199 g). On day 30, the mean sleeping times for pre-tolerant, tolerant and post-tolerant animals were 187.0 ± 9.2, 88.0 ± 4.7 and 213.5 ± 5.3 minutes. The tolerant group slept for a significantly shorter ($P = 0.01$), and the post-tolerant group for a significantly longer ($P = 0.02$), time than the pre-tolerant animals.

Pentobarbital blood level studies. No sleeping times were obtained from rats sacrificed for cardiac puncture. However, the tolerant and post-tolerant groups received injections of pentobarbital prior to the day of sacrifice, and sleeping times were measured in these cases. The mean sleeping time for 68 rats receiving a first dose of the barbiturate was 200.0 ± 6.05 minutes. For 34 rats receiving a second dose of the drug 24 hours after an initial dose, the mean sleeping time was 104.0 ± 3.70 minutes. A significant difference between these two mean values was found ($P = 0.01$), establishing the development of tolerance in these animals.

TABLE III. Mean Whole Blood Levels (μg/ml) $\pm$ S.E. of Pentobarbital in Female Rats at Various Times Following Administration of 30 mg/kg i.p.

Group	Time (min) following injection of pentobarbital				
	15	30	60	120	180
Pre-tolerant	24.9 $\pm$ 3.1 (5)*	19.1 $\pm$ 3.3 (5)	14.3 $\pm$ 2.7 (5)	11.7 $\pm$ 1.6 (5)	11.1 $\pm$ 1.8 (5)
Tolerant	28.1 $\pm$ 4.6 (5)	23.7 $\pm$ 4.5 (5)	14.6 $\pm$ 2.4 (5)	4.8 $\pm$ 2.0 (5)	1.3 $\pm$ .2 (5)
Post-tolerant	28.0 $\pm$ 4.2 (6)	17.8 $\pm$ 1.5 (6)	16.7 $\pm$ 2.1 (6)	12.0 $\pm$ 2.4 (6)	7.2 $\pm$ .2 (5)

* No. of animals.

Table III lists the mean whole blood barbiturate levels obtained in 79 rats sacrificed at various times following administration of 30 mg/kg pentoparbital i.p. These same data are presented in Fig. 1, relating blood barbiturate level to the logarithm of time. Blood levels of the tolerant and post-tolerant groups were not influenced by prior barbiturate treatment as evidenced by the fact that, in 5 rats, no measurable amount of drug was found in blood 24 hours following administration of 30 mg/kg pentobarbital i.p. Inspection of Fig. 1 indicates that the rate of metabolism of pentobarbital was increased in tolerant rats, but unchanged in post-tolerant rats, compared to the pre-tolerant group. This was substantiated by application of the 'Student' t-test to the data in Table III, which revealed a significant difference between mean barbiturate blood levels only in the case of the pre-tolerant and tolerant rats at 120 ($P = 0.05$) and 180 minutes ($P = 0.02$).

Discussion. This investigation has demonstrated the development of a delayed hypersensitivity to pentobarbital in the rat which is, however, statistically significant ($P = 0.05$) only in the case of the female. The relative increase in sleeping time in the female (14.2%) is greater than in the male (11.0%). This is correlated with a relatively greater degree of tolerance and a relatively higher initial sensitivity to pentobarbital in the female than in the male animal These results corroborate previous reports of post-tolerance hypersensitivity in which pentobarbital, administered to male rats 10 to 12 days following acute induction of tolerance, was found to be 14% more potent than in naive animals(2). It was suggested, in this latter report, that such a phenomenon could be related either to an increase in sensitivity of those central nervous system elements responsive to the drug or, alternatively, to a depletion of precursors of barbiturate-metabolizing enzymes in liver consequent to the accelerated synthesis of these enzymes reported to occur during tolerance.

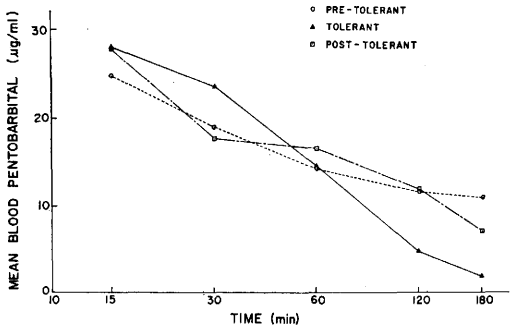

FIG. 1. Relationship between mean blood pentobarbital level (μg/ml) and time (log min) elapsed since administration of 30 mg/kg sodium pentobarbital i.p. to pre-tolerant, tolerant, and post-tolerant groups of female rats.

It was found, in the present study, that after a fixed dose of pentobarbital, the barbiturate blood level declined more rapidly in tolerant than in pre-tolerant female rats. This corroborates the observations of other workers(3,4) who have shown tolerance induction to be correlated with increased hepatic enzyme activity. The rate of decline of barbiturate blood levels in post-tolerant female rats, however, did not differ from that seen in the pre-tolerant group, indicating that an alteration in rate of metabolism of the drug was not responsible for the increased sleeping time observed. This conclusion was further borne out by the observation that in these female rats on day 30, the mean wet liver weight (expressed as % body weight) in the post-tolerant (3.49 $\pm$ 0.08) did not differ significantly from that in the pre-tolerant group (3.58 $\pm$ 0.11), while that in the tolerant group (3.78 $\pm$ 0.10) was significantly increased. It appears, therefore, that the ob-

served delayed hypersusceptibility to pentobarbital is the result of an increase in the sensitivity of the central neuronal systems responsible for the anesthetic effects of the drug. These results, of course, do not rule out other possible mechanisms of hypersensitivity, such as alterations in rate of permeation of the blood-brain barrier by pentobarbital.

Summary. Rats receiving a fixed anesthetic dose of pentobarbital developed hypersensitivity to the drug 28 days following tolerance induction. This delayed increase in sleeping time was non-significant in the male, but significant in the female animal ($P = 0.05$). Tolerant female rats showed an increase in liver weight (as % body weight) and an enhanced rate of disappearance of barbiturate from blood. Hypersensitive female rats failed to exhibit a significant change either in liver weight on in rate of decrease of barbiturate blood level compared to naive animals. Delayed hypersensitivity to pentobarbital in the female rat appears, therefore, to be unrelated to alternations in hepatic metabolizing enzyme activity.

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Response of Heart Rate, Oxygen Consumption, and Arterial Blood Pressure to Graded Exercise in Dogs.* (30847)

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In man the relationship of heart rate to oxygen consumption during exercise is sufficiently direct that an increase in heart rate may be used as an objective measure of an increase in work load(1). It would be of value to know if the same direct relationship obtained in the dog since heart rate can be measured easily and without disturbing the animal. In addition, since there are few reports in the literature on the effect of graded exercise on arterial blood pressure in the dog, it was thought worthwhile to attempt this measurement in a reasonably large number of animals.

Methods. Dogs were trained to exercise on the treadmill until they were thoroughly accustomed to the procedure and would run on the moving platform without being attached by a leash to the apparatus. A complete exercise study consisted of a continuous run at grades of 0, 7, 14, 21, and 28% at 5.5 km/hr, for a period of 5 minutes at each work level. Measurements of heart rate, oxygen consumption, and aortic blood pressure were made during the period of exercise.

Heart rate was recorded at rest, throughout exercise, and during recovery by a direct-writing electrocardiograph coupled to an anterior-posterior lead.

Oxygen consumption was determined by collection and analysis of expired air. A permanent tracheostomy(2) was constructed to permit insertion into the trachea of a cuffed piece of Tygon tubing (⅜ to ½ inch I.D.) attached to a glass U tube bearing inlet and outlet valves (Collins J type valve). The U tube fitted snugly round the dog's neck and the unit was carried easily by the dog. The volume of air expired into a latex

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