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Developmental and Behavioral Effects of Prenatal Primidone Exposure in the Rat

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PIZZI, W. J., T. D. ALEXANDER AND J. T. LOFTUS. *Developmental and behavioral effects of prenatal primidone exposure in the rat.* PHARMACOL BIOCHEM BEHAV **55**(4) 481–487, 1996.—Pregnant Sprague-Dawley rats were administered primidone (PRM) by oral gavage on gestation days 8–17 in doses of 0, 40, and 80 mg/kg. Although these doses of PRM did not produce significant differences in litter size, birth weight, mortality, date of attainment of developmental landmarks or measures of preweaning reflex and motor development, there were a number of significant differences that developed as the animals approached and entered adulthood. When tested as adults, the 80 mg/kg male rats showed a deficit in the performance of an eight-arm radial maze task. These same animals showed a significant reduction in open field activity when tested as adults. In addition, both male and female PRM-treated animals showed reduced body weights at different periods corresponding to onset of sexual maturation during development. These findings are consistent with the larger body of literature reporting on the neurobehavioral teratology of phenobarbital, including its ability to produce lesions in the hippocampus and endocrine dysfunction resulting in reproductive deficits. These results suggest that PRM produces its adverse effects as a result of its metabolism to phenobarbital, which in turn affects the limbic system.

Primidone Radial arm maze Neurobehavioral teratology Phenobarbital Open field activity

THE search for a safe antiepileptic drug in the treatment of pregnant epileptics remains an elusive goal. Although a number of effective antiepileptic drugs (AEDs) can be used to treat the mother, these all carry a risk for the fetus. All of the major AEDs have been associated with teratogenesis (5,33). A number of AEDs have been implicated as behavioral teratogens. Phenytoin has been reported to be a behavioral teratogen in humans (10,11) and animals (27,37,38). Valproic acid has also been reported to be teratogenic in humans (1,3) and animals (39). Valproic acid also produces behavioral teratogenicity in animal studies (4,40). Carbamazepine (28) does not appear to be a potent behavioral teratogen; however, this may be a moot point because both carbamazepine and valproic acid have been associated with a 1–2% increased rate of spina bifida (2,31). Phenobarbital (PB) has been the most frequently prescribed barbiturate in the treatment of epilepsy, and primidone (PRM) is often prescribed as an adjunct therapy. PB and PRM have been implicated in teratogenesis and behavioral impairment in humans and animals (17,24,34,35). Although a number of animal studies have found that in utero exposure to PB results in learning deficits (21), we know of no similar studies with PRM.

The fact that PRM is metabolized to PB suggests that the findings from studies of in utero exposure to PB may predict the outcome of PRM exposure. Murai (23) found a greater number of errors on the Hebb-Williams maze among mice exposed to PB in utero. Martin et al. (15,16) reported a number of learning or performance deficits in rats exposed in utero to 40 or 80 mg/kg PB, including fewer escapes on a two-way conditioned avoidance task, fewer escapes in a Sidman avoidance task and less efficient performance on a differential reinforcement of low rates of responding (DRL) operant schedule.

Middaugh et al. carried out a number of operant conditioning experiments in mice prenatally exposed to PB, and they demonstrated an alteration of behavior maintained by food reinforcement. One of their tasks was a progressive-fixed-ratio schedule in which the animal is given a food reward for a fixed number of bar presses, with the fixed number of presses increasing in large steps (e.g., 40,80,120,160). PB-exposed offspring showed a decreased response rate during the increased work demand (18,19). In these studies, the maternal dose levels were 20, 40 or 80 mg/kg. Two other studies have reported impairments of fixed-ratio performance (13,15).

Although no specific physiological mechanism has been

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found to be directly responsible for the behavioral changes following prenatal exposure to PB, various brain lesions, changes in brain monoamine levels and endocrine hormones may have a critical role in these deficits. Middaugh et al. (20) and Fishman and Yanai (6) reported altered monoamine neurotransmitter levels following in utero exposure to PB. Fishman and Yanai (6) showed that high prenatal blood levels of PB (100 μg/ml) can cause a reduction in cerebellar Purkinge cells and hippocampal pyramidal cells. The finding that PB can cause damage to the cerebellar Purkinge cells has been reported by others (9). Fishman and Yanai (6) also showed that neonatal subcutaneous administration of 40–50 mg/kg of PB on postnatal days (PNDs) 2–21 can damage cerebellar Purkinge and granule cells along with hippocampal pyramidal and granule cells. Many of the learning deficits reported for PB-exposed animals are similar to those seen in rats with hippocampal lesions, including deficits in avoidance learning, maze learning and inefficient DRL performance.

An important consequence of in utero exposure to barbiturates is the effect that these drugs have on endocrine physiology. Research in rats has shown that barbiturates alter both the pituitary–adrenal and pituitary–gonadal axes (7,8,30). The modification of endocrine function in rats occurs early in development and continues to play a role as various hormone-mediated functions mature. The effects on pituitary–adrenal physiology may play a role in the learning and performance deficits. The pituitary–adrenal axis influences behavioral performance in a number of areas, specifically open field activity and active and passive avoidance behavior (25). Gupta et al. demonstrated the effects of PB on the pituitary–gonadal axis (7,8). In these studies, both female and male rats exposed to 40 mg/kg PB during gestation showed delays in the onset of puberty and reduced fertility as adults.

PRM is metabolized into two active pharmacological agents; namely, PB and phenylethylmalonamide (PEMA). Because PEMA is reported to only have 20% of the potency of PB's antiepileptic activity and to carry less neurotoxicity (36), the present study was undertaken to determine whether PRM would produce neurobehavioral teratology similar to that seen with other barbiturates.

METHODS

Subjects

Subjects were 50 sperm-positive (gestation day [GD] 0), nulliparous Sprague-Dawley rats obtained from the Holtzman Farms (Madison, WI). Pregnant dams were randomly assigned to treatment groups on GD 5 and individually housed in polycarbonate cages with ad libitum access to pelleted rat chow and tap water. All animals were maintained on a 12-h light/dark cycle for the duration of the study.

Treatment

The dams were administered 5-ethyl-5-phenylhexahydropyrimidine-2,4-dione (Primidone, Sigma, St. Louis, MO) mixed in a corn oil vehicle by oral gavage once daily from GD 8 to GD 17. The treatment groups consisted of a vehicle control (VC; $n = 16$), and PRM groups at doses of 40 mg/kg (PRM 40, $n = 17$) and 80 mg/kg (PRM 80, $n = 17$). The group sizes were reduced to 15, 11 and 13 for the VC, PRM 40 and PRM 80 groups, respectively. One VC group did not meet the group composition criteria of eight pups (4 ± 1 of each sex). The PRM 40 group contained one female that was not pregnant, three with resorption sites, and two litters that did not meet the group composition criteria for inclusion in the study. The PRM 80 group contained one female that was not pregnant, one with resorption sites, and two litters that did not meet the group composition criteria for inclusion in the study. Upon delivery (PND 0), pups were counted, weighed, sexed, examined for gross anatomical abnormalities and randomly culled to litters of eight (4 ± 1 of each sex). For identification, each pup had its paws tattooed with a commercial tattoo ink. Pups were weighed every 5 days to PND 30 and checked daily for mortality. Pups were weaned at PND 24 and housed separately by sex (two per cage).

Developmental Landmarks

Developmental screening employed one male and one female from each litter for each landmark and behavioral measure. The assignment of the animal to be tested on each task was done randomly prior to the start of the experiment, with some of the animals being tested on several measures. The unit of analysis for all preweaning data was the day of attainment of criterion, with the exception of pivoting locomotion. A brief description of each measure follows.

Pinna detachment. Beginning on PND 2, pups were inspected daily for the complete separation of the pinnae from the cranium. Prior to detachment, the distal portion of the pinna is folded over the auditory meatus, and detachment was defined as the pinna being raised to a position of less than 90° from its final position.

Incisor eruption. Beginning on PND 7, pups were inspected daily for the emergence of both the upper and lower incisors from the gingiva. Criterion for this measure was the day on which both upper and lower incisors emerged from the gingiva.

Eye opening. Beginning on PND 9, pups were inspected daily for the complete opening of both eyelids.

Testes descent. Beginning on PND 21, male pups were inspected daily for complete descent of both testes into the scrotum.

Vaginal patency. Beginning on PND 30, female pups were inspected daily for invagination and opening of the vaginal orifice.

Preweaning Reflex and Motor Development

Surface righting. Beginning on PND 3, pups were given two trials daily on the surface-righting task. The pup was placed on its back and was considered to have righted itself when all four paws were under the animal. Criteria for this task required the animal to right itself for two consecutive trials in less than 3 s per trial on 2 consecutive days.

Negative geotaxis. In this task, the pup was placed head down on a 25° inclined plane that had a surface covered in fine-grade sand paper, and the latency to turn 180° to the head-up position was recorded. Criterion behavior was completion of the 180° turn within 45 s. Unsuccessful animals were scored as falling, turning less than 180° or bracing. Pups were tested on PNDs 7–9.

Pivoting locomotion. On PNDs 7–9, the total number of degrees turned by the pup (in either direction) during a 1-min period was recorded. This task was performed in a circular metal container (24.5 cm in diameter, with 5-cm-high walls) lined with a brown paper insert on which lines had been drawn to delineate the four 90° quadrants. The number of degrees turned was scored in completed 90° segments, and any movements within a quadrant or retracing of movements in less than 90° segments were ignored. The use of a circular metal

container allows the animal to make some forward movements without being touched by the experimenter, while keeping the animal within the testing field.

Behavioral Testing in Adult Animals

Open field activity. Open field activity was measured in a large open field (87 × 87 cm) apparatus that was transected by 12 light beams (6 on each axis) arranged to form a grid pattern with 49 squares. The measure of activity was the total number of light beams broken per 5-min test period. The male animals were tested on PND 84 and the female animals on PND 86. There were 10 subjects in each treatment group, with only one male and one female selected from each litter. All testing was done with ambient lighting from overhead flourescent fixtures.

Eight-arm radial maze. The maze was constructed of black plexiglass with the following dimensions. The running surface of the maze was raised 58 cm above the floor and consisted of a central octagonal platform measuring 33 cm in diameter, with extended arms that were 46 cm long and 10 cm wide. Each arm had walls that were 4.5 cm high and a bait well that was 1.5 cm in diameter, 0.5 cm deep and located 43 cm from the beginning of the arm. The testing room was well lighted and contained numerous external visual cues. Ten male and 10 female pups from each treatment level were tested, with only one pup of each sex from the same litter. Because this study used a completely randomized design, the first 10 litters in each treatment group were used as a representative sample in this labor-intensive task.

Males began testing at 83 days of age and females began testing at 125 days of age.

Prior to testing, all animals had been on an ad libitum feeding schedule and were weighed to determine their free-feeding body weights. Each animal's food intake was then limited to reduce its free-feeding body weight to 85%. During the testing period, animals were weighed daily and fed an adjusted portion of food to maintain the 85% weight criterion. Each week, 5 g were added to each animal's free-feeding weight to account for normal weight increases. Prior to the actual testing, each animal was introduced to the maze for a 5-min habituation period on each of three consecutive days. On the first day, the food rewards to be used in this task (Froot Loops® cereal) were scattered randomly throughout the apparatus. On day two, the rewards were only placed in the arms of the maze, and on day three only at the goal end of each arm. Following this introduction to the maze, the animals were tested for 5 consecutive days, followed by a 2-day break and another 10 consecutive days of testing. Each animal was given one test trial per day, which consisted of placing the animal in the center of the maze and allowing it to move freely about the maze to retrieve the food rewards (one-fourth piece of a Froot Loop) from the food wells. Each animal's performance was recorded and scored for the number of correct arm choices and for the pattern of choices for the entire trial. A correct choice was defined as an entry into any arm that had not been previously entered. An error was defined as any reentry into a previously visited arm of the maze. The criteria for completion of the task were retrieval of all eight baits, a maximum of 16 arm entries or a maximum of 5 min in the maze on a given trial. The criterion for entry into an arm was the placement of all four feet into the arm.

During the final five days of testing, performance was assessed to determine if the animals were using a response patterning strategy. The procedure used was that reported by Harrell et al. (12) and consisted of scoring the animals' responses in a clockwise pattern from the previous choice as +1 through +4 or counterclockwise from the previous choice as −1 through −3. The tendency for an animal to use a particular response strategy was evaluated by summing choices over days for the second through eighth choices. Animals consistently choosing an adjacent arm in a particular direction (choices scored as +1 or −1) or every third arm (choices scored as +3 or −3) could obtain all the rewards without reentering an arm and without having to make a spatial discrimination. Animals using the 1 or 3 choice strategy for 40% or more of the choices were classified as using a nonspatial response patterning strategy, and all other choice patterns were classified as spatial strategies.

Data analysis. A one-way analysis of variance was used for all parametric data, and all post hoc testing used the Bonferroni *t* test. Nonparametric data were analyzed with the chi-square test. Data were processed by using the InStat program from GraphPad (San Diego, CA).

TABLE 1

MEAN (SEM) MATERNAL AND PUP BODY WEIGHT (g) VALUES

	Treatment		
	VC	PRM-40	PRM-80
Maternal weight gain, GD 8–18	78.8 (3.2)	78.4 (3.3)	79.0 (3.7)
n	16	13	15
Pup body wt., PND 2			
Females	10.0 (0.1)	10.0 (0.3)	9.5 (0.3)
Males	10.5 (0.1)	10.2 (0.3)	10.1 (0.3)
n	16	13	15
Pup body wt., PND 25			
Females	102.3 (1.3)	98.6 (2.3)	95.0 (1.3)*
Males	108.3 (1.4)	101.1 (3.2)	102.1 (1.7)
n	15	10	13
Pup body wt., PND 30			
Females	134.7 (1.5)	130.5 (2.6)	126.1 (1.4)*
Males	149.7 (1.5)	142.5 (3.7)	142.3 (2.2)
n	15	10	13
Pup body wt., PND 50			
Females	235.2 (2.7)	227.3 (5.1)	217.4 (2.5)*
Males	331.4 (3.1)	321.5 (6.2)*	320.0 (4.8)*
n	15	10	13
Pup body wt., PND 91			
Females	310.5 (3.7)	304.0 (6.5)	295.0 (4.3)
Males	542.5 (10.2)	519.9 (20.4)	530.2 (11.4)
n	15	10	13

*$P < 0.01$.

RESULTS

Maternal and Developmental Effects

The dosages of PRM used in this study did not result in any maternal weight reductions (Table 1). Although there were no differences in the birth weights of PRM-exposed pups, weight differences did emerge during the postnatal period, with both the PRM-40 and PRM-80 groups showing a decrease in body weights when compared with controls. These weight differences began in the PRM-80 females at PND 25 and were present in the PRM-40 and PRM-80 males by PND 50 (Table

TABLE 2
DEVELOPMENTAL LANDMARKS
(MEAN DAY OF ATTAINMENT)

	Treatment		
	VC (n = 15)	PRM-40 (n = 10)	PRM-80 (n = 13)
Pinnae			
Females	2.3 (0.1)	2.2 (0.1)	2.5 (0.2)
Males	2.3 (0.1)	2.5 (0.2)	2.3 (0.1)
Incisor			
Females	9.8 (0.2)	9.7 (0.2)	10.3 (0.2)
Males	10.0 (0.2)	9.6 (0.3)	10.1 (0.3)
Eye			
Females	14.4 (0.2)	14.3 (0.1)	14.8 (0.2)
Males	14.7 (0.2)	14.4 (0.2)	14.5 (0.1)
Testes descent			
Males	26.3 (0.3)	25.9 (0.2)	25.8 (0.2)
Vaginal patency			
Females	34.8 (0.5)	34.6 (0.6)	35.5 (0.5)

Numbers in parentheses signify the standard error of the mean.

1). However, the body weights returned to control values by PND 91.

There were no significant differences between any of the PRM-exposed offspring and control animals on a series of developmental landmarks. Table 2 shows the means for day of attainment for pinna detachment, incisor eruption, eye opening, vaginal patency and testes descent.

Preweaning Reflex and Motor Development

Table 3 shows the results from the surface righting, negative geotaxis and pivoting locomotion tasks. None of the measures showed significant differences.

Adult Testing

Figure 1 shows that the PRM-80 males had a significantly reduced level of open field activity (F = 4.76, p < 0.02). Neither the PRM-40 male group nor the female drug-exposed groups differed significantly from their appropriate control groups.

TABLE 3
TESTS OF PREWEANING REFLEXES
AND MOTOR DEVELOPMENT

	Treatment		
	VC (n = 15)	PRM-40 (n = 10)	PRM-880 (n = 13)
Surface righting (Mean age of attainment)			
Females	7.0 (0.4)	7.0 (0.3)	7.3 (0.5)
Males	6.1 (0.3)	6.2 (0.4)	5.6 (0.2)
Negative geotaxis (Mean seconds to completion over 3 test days)			
Females	20.4 (2.8)	16.9 (1.4)	24.9 (2.9)
Males	21.0 (2.3)	20.0 (2.8)	19.8 (2.9)
Pivoting locomotion (Mean 90° segments traversed over 3 days)			
Females	7.7 (0.7)	8.9 (1.2)	5.6 (0.9)
Males	9.8 (1.4)	11.3 (1.3)	9.5 (0.9)

Numbers in parentheses signify the standard error of the mean.

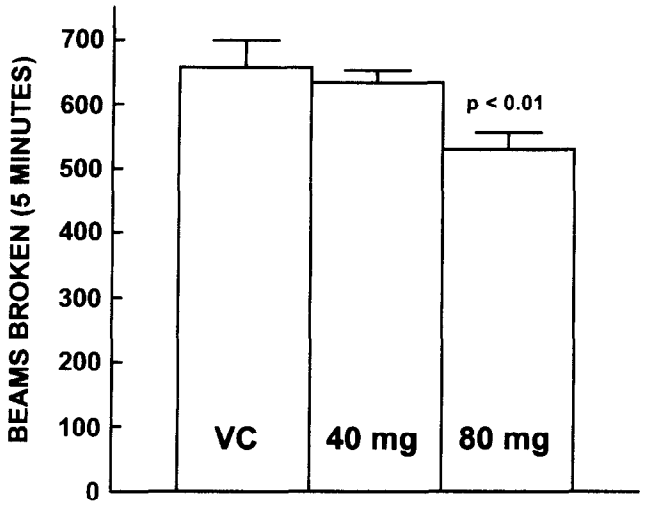

FIG. 1. Open field activity of male rats. Mean (±SE) number of beam breaks over a 5-min test period for male rats on PND 84.

The male PRM animals also showed significant decreases in performance on the eight-arm radial maze. The PRM-80 male offspring showed a deficit in the number of correct choices out of the first eight-arm entries; however, no deficits were seen in the PRM-40 male group (Fig. 2). No differences were seen in any of the female exposed groups. When an overall analysis of all 15 trials was done, the PRM male groups showed a significant treatment effect (F = 4.475, p < 0.05); however, there was no treatment effect in the PRM female animals. A post hoc analysis showed that the PRM-80 male animals made fewer correct choices than did controls (p < 0.02) or the PRM-40 males (p < 0.02). Because the first 5 days of testing were followed by a 2-day recess and then 10 consecutive days of testing, the last 10 days were analyzed separately. Again, a significant treatment effect was seen in

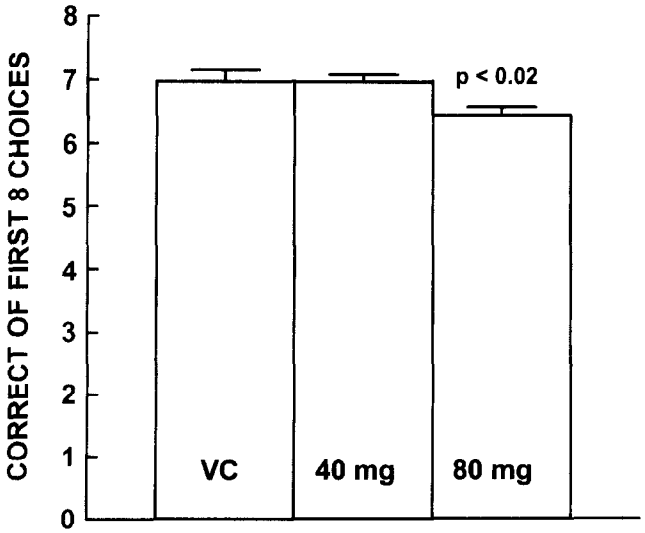

FIG. 2. Overall performance of male rats on the eight-arm radial maze. Mean (±SE) number of correct arm entries out of the first eight choices averaged over the 15-day test period on the eight-arm radial maze.

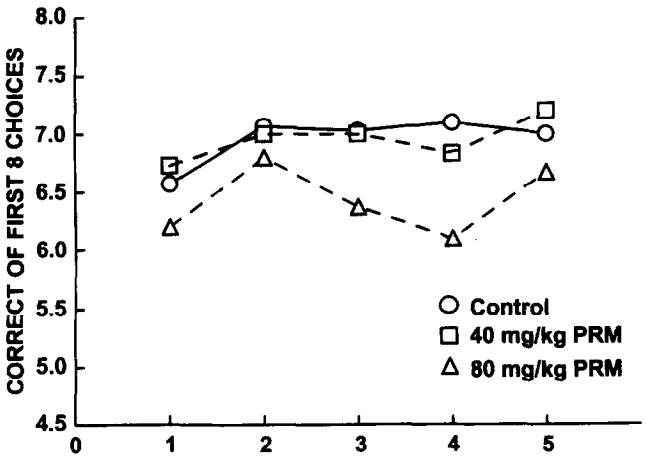

FIG. 3. Performance of male rats plotted in 3-day blocks on the eight-arm radial maze. Mean number of correct choices by male rats plotted as blocks of 3 days over the 15-day test period on the eight-arm radial maze. Triangles = PRM-80, squares = PRM-40, circles = VC.

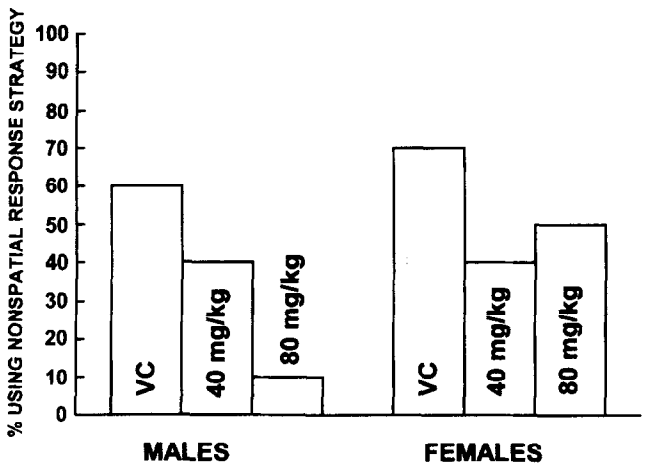

FIG. 4. Analysis of response strategy used on the eight-arm radial maze. The percentage of responses in which a nonspatial response strategy was utilized by males and females in each of the treatment groups.

the males ($F = 4.55$, $p < 0.02$) but not in the females. The post hoc analysis again showed the PRM-80 male group to be significantly different from both the controls ($p < 0.02$) and the PRM-40 group ($p < 0.02$). Figure 3 shows the performance of the male animals across the 15 days of testing plotted in blocks of three trials or days.

The radial arm maze data were further analyzed for the use of a response patterning strategy reported by Harrell et al. (12). This analysis evaluated the performance of subjects during the final 5 days of testing to determine if the pups were navigating the maze through the use of extramaze visual cues (spatial strategy) or through the use of a response patterning strategy (nonspatial strategy).

By using the criteria set by Harrell et al. (12), animals consistently responding +1 or −1 +3 or −3 for 40% or more of the choices were designated as using a nonspatial response patterning strategy. An overall chi-square analysis of the response patterns for all groups was performed and found to be significant ($\chi 2 = 86.9$, $p < 0.0001$). This analysis was followed by post hoc comparisons in which all drug-treated animals used a nonspatial response strategy significantly less often than did their respective control groups ($p < 0.01$).

When the PRM-80 male animals with a deficit on the eight-arm radial maze were compared with the PRM-80 female animals that did not show a deficit, there was a significant difference in their use of the spatial response strategy. Specifically, the males used a nonspatial strategy significantly less often than did the females ($\chi 2 = 36.2$, $p < 0.001$). In this analysis, 10% of the male rats used a response patterning strategy and 90% used a spatial strategy. The female animals split 50/50 with respect to a spatial versus nonspatial strategy (Fig. 4).

DISCUSSION

The results of this investigation show that offspring of rats exposed to PRM at doses of 40 and 80 mg/kg show no behavioral deficits on the indices of reflex and motor development tested for prior to adulthood. The adult male animals exposed to PRM-80 showed a significant impairment in performance on the eight-arm radial maze, a behavioral task sensitive to memory impairments (12). Specifically, our male drug-treated animals (PRM-80) failed to make as many correct choices as control animals out of their first eight-arm entries. Further analysis showed that these males were using a nonspatial response strategy significantly less often than their respective control group, as well as female animals treated with the same PRM dose that did not show a deficit on the eight-arm radial maze task. The fact that there were no differences on the eight-arm radial maze performance between PRM-40-treated and control males or between PRM-40-treated male and female animals suggests that adoption of a different strategy is not a dimorphic sex response. Such an inefficient response strategy is consistent with the types of learning behavior seen in animals with limbic lesions and concomitant memory impairment (12). All PRM-exposed groups showed a significant reduction in the use of the nonspatial response strategy. Although we cannot say with any certainty what precise cognitive deficit exists in these animals, further studies with the eight-arm radial maze may clarify the nature of the impairment.

Our data are also consistent with the findings for the neurobehavioral toxicology and teratology of PB. Fishman and Yanai (6) reported that PB results in hippocampal damage, and Pick and Yanai (26) showed that PB exposure results in deficits in eight-arm radial maze performance. A recent report by Rogel-Fuchs et al. (32) extended the earlier findings by Fishman and Yanai (6) to show that mice exposed either prenatally or neonatally to PB showed learning deficits on the Morris water maze. This maze, like the eight-arm radial maze, is sensitive to lesions in the septohippocampal system, which disrupt memory and/or spatial orientation (22). A biochemical analysis of the septohippocampal area showed an increase in maximal receptor number (Bmax) for muscarinic cholinergic receptors and an increased formation of inositol phosphate following carbachol stimulation (6). These changes and the failure to find a change in choline acetyltransferase led them to conclude that the cholinergic changes are postsynaptic in nature.

Recent findings following prenatal oxazepam exposure in mice suggest that GABAergic drugs modulate cholinergic projections to the hippocampus, leading to memory impairments such as those seen in the eight-arm radial maze (14). These investigators found that prenatal oxazepam exposure pro-

duced a long-term increase in cholinergic receptor number and binding affinity, which they posited leads to exaggerated behavioral responses and consequently to performance deficits. They also reported an increase in neophobia (measured as an increased latency to approach a novel stimulus) but no differences in open field exploratory activity. PRM and PB probably also act on this system to produce similar interactions with hippocampal cholinergic neurotransmission. The data from prenatal oxazepam exposure, the data showing that PB can cause structural and neurochemical changes in hippocampus (26,32) and the results of the experiments reported here are consistent with a developmental neurotoxic mechanism affecting the septohippocampal system, although this mechanism is not limited to these brain areas.

The growth curves seen in the present study are consistent with a perturbation of the endocrine system, perhaps caused by lesions in the limbic–hypothalamic system. The PRM-40 and PRM-80 male rats showed a significant decrease in body weight at PND 50, when systematic weight measures ceased, so that various behavioral measures involving food deprivation could be performed. A similar pattern of decreased weight gain was seen in female rats starting at PND 25 for the PRM-80 group and continuing through PND 50. These body weight differences may coincide with maturation of the endocrine system in the rat. That these decreased weights may indicate an endocrine deficiency is further supported by the reports of Gupta et al. (7,8) that 40 mg/kg of PB administered to pregnant rats aged GD 12–19 suppressed body weight gains and impaired reproductive functioning in the offspring.

PB and PRM may play a role in endocrine development through more than one mechanism. One mechanism may be through a direct neurotoxic effect as suggested by the studies reviewed above. A second mechanism may involve the ability of these drugs to induce enzyme production in the liver, with a subsequent greater metabolism of circulating hormones during a critical period of development (29,30). This second mechanism would be of particular interest with respect to adult reproductive functioning and to the development of sexually dimorphic behaviors.

PRM is converted into two active metabolites: PB and PEMA. The parent drug and both metabolites have antiepileptic activity; however, PEMA is reported to have 20% of the potency of PB (36). Although PRM is not strictly categorized as a barbiturate, it is clear that many of its effects are the result of its metabolism with PB. The findings in this study, including deficits in the radial arm maze, decreased open field activity and body weight differences, are consistent with the findings reported in the literature, thus demonstrating that prenatal exposure to PB results in neurotoxic effects in the brain, including but not limited to the septohippocampal system. Our working hypothesis is that the functional teratology resulting from prenatal exposure to PRM is largely the result of its metabolism to PB.

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