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Behavioral and Developmental Effects in Suckling Mice Following Maternal Exposure to the Mycotoxin Secalonic Acid D

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BOLON, B. AND V. E. V. ST. OMER. *Behavioral and developmental effects in suckling mice following maternal exposure to the mycotoxin secalonic acid D.* PHARMACOL BIOCHEM BEHAV **34**(2) 229–236, 1989.—Pregnant mice (dams) were gavaged once on gestational day 13 with 4 ml/kg of dimethylsulfoxide vehicle containing 0 (groups 15, 25 and negative control) or 25 (positive behavioral teratogenic control group) mg/kg of secalonic acid D (SAD). While nursing their offspring, dams were gavaged on postgestational days 1 to 10 with vehicle containing 0 (negative and positive control groups), 15 (group 15) or 25 (group 25) mg/kg/day of SAD. Gestational lengths, maternal pregnancy weights, litter sizes, neonatal sex ratios, neonatal physical appearance and female birth weights were unaffected by prenatal treatment, but male pups born to positive control dams weighed less ($p<0.05$) than negative control group. Compared to negative control, the positive control dams gained significantly more weight while nursing their offspring. Prenatal (positive control) and postnatal (15,25) SAD exposure delayed ontogeny of surface righting, olfactory discrimination and hindlimb grip behaviors in males and females, and testes descent in males. Negative geotaxis in male and female offspring of group 25 and male offspring of positive control group, as well as times of incisor eruptions of both sexes in groups 15 and 25 were delayed. A significant dose-response effect in olfactory discrimination existed between the groups exposed to postnatal SAD. SAD was behaviorally teratogenic following both prenatal and early postnatal exposure.

Mycotoxin Secalonic acid Prenatal-postnatal exposure Developmental neurotransmitters Behavioral alterations Nursing mice

SECALONIC acid D (SAD), an environmental contaminant, is a major toxic metabolite of several fungal species, notably *Penicillium oxalicum* (32). Penicillia may infect approximately 44% of all preharvest corn samples in the midwestern United States (7). Levels of SAD ranging from 0.3 to 4.5 ppm have been found in corn dust from several midwestern and southern grain elevators (10). Therefore, ingestion of food or inhalation of grain dust contaminated with SAD may be a common occurrence in humans (8,35).

Data on the fetotoxicity, structural teratogenicity, acute and chronic toxic effects of SAD in several species are limited (19, 23, 24, 31, 32), and the occupational hazards of SAD to humans are speculative (35). Although documented episodes of human intoxication have not been reported, experimental animal research suggests that SAD may be a potential toxicant to both developing and mature humans (8, 19, 22–24, 31).

An important issue regarding the toxic effects of mycotoxins concerns their effects on neurobehavioral development of mammalian progeny following early exposure to doses lower than those associated with visible birth defects. It is obvious that structural defects alone (e.g., death, growth retardation and gross malformations) following prenatal or early postnatal exposure to nonmalforming or nonobservable effect levels (NOEL) of SAD are insufficient indicants of toxicity. The developing brain is especially vulnerable to insults induced by chemical agents that cross the blood-brain barrier during critical periods of development (4,33). The only reported behavioral teratology study to date indicates that SAD readily crosses the mammalian placenta since in utero exposure to NOEL of SAD causes certain behavioral deficits in preweaned mice (29). However, exposure of the brain to NOEL of neurotoxins during the early postnatal critical periods of development may also induce subtle or overt neurochemical and neurobehavioral alterations in species (e.g., human, mouse) which are developmentally immature at birth (4, 16, 27). Limited animal experimental data indicates that the administration of drugs to nursing mothers induces neurobehavioral and neurochemical def-

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icits in the suckling offspring (18, 28, 30). However, the neurobehavioral effects in the developing mammalian progeny following early postnatal exposure to SAD are not known. With the increasing proportion of women returning to the work force soon after childbirth, the potential exists for the absorption of occupational chemicals by nursing mothers with secretion into breast milk and transfer to nursing infants. More information about the susceptibility of nursing mammals following exposure of the lactating mothers to environmental toxins is thus urgently required. This study was conducted primarily to assess the maturational and behavioral effects on developing mice following early postnatal exposure to SAD via the dams' milk.

METHOD

Animals and Basic Design

Impregnated nulliparous CD-1 mice (age 56–63 days) were kept in rooms having a constant light-dark cycle (light, 5:00 a.m. to 7:00 p.m.), temperature (22–23°C) and relative humidity (40 ± 10%). Water and Purina Rat Chow (Ralston-Purina, St. Louis, MO) were available ad lib. Females were randomly assigned to one of four treatment groups at conception. The presence of a vaginal plug indicated gestational day (GD) 0. Animals from each treatment group were housed in sets of four from GD0 to 15 and then placed in individual cages. Dams were weighed on GD7, 12, and 18 and on postgestational days (PGD) 1, 10 and 21. Dams were observed daily from GD1 to PGD22 for behavioral abnormalities and necropsied on PGD22.

Pregnant mice delivered normally. Deliveries completed by 5:10 a.m. were designated as postnatal day (PND) 1. Pups born later that day were designated PND1 on the following day. Offspring in each litter were counted, weighed, sexed, examined for deaths and external malformations, culled to 8 pups of equal number of sexes and foot marked (Nos. 1–4, males; Nos. 5–8, females) on PND1. Pups were weaned on PND22. Post-mortem examinations performed on all dead pups revealed no gross malformations. Offspring were nursed by their biological dams. The evaluation of maternal weight gain, length of gestation and reproduction outcome were based on examination of all dams used to produce 8–11 litters/treatment group. Two males (Nos. 3, 4) and two females (Nos. 7, 8) from each litter were tested for physical and behavioral development as described below. Culled offspring (PND1) and behaviorally untested offspring (Nos. 1, 2, 5, 6) from each litter were sacrificed on PND1, 7, 10, 13 or 16, respectively, between 6:40 to 6:50 a.m. by immersion in liquid nitrogen. Gastric contents and frontal cortex from culled pups and behaviorally untested pups sacrificed on PND10 were removed at approximately 0°C and stored at −70°C until assayed for SAD. Brains from behaviorally untested pups were used for a separate neurochemical study (5).

Dosing

Pregnant mice (dams) were gavaged once on GD13 with 4 ml/kg of dimethylsulfoxide (DMSO) vehicle containing 0 (groups 15, 25 and negative control group N) or 25 (positive behavioral teratogenic control group P) mg/kg of SAD. While nursing their offspring, dams were gavaged on postgestational days (PGD) 1 to 10 with vehicle containing 0 (groups N, P), 15 (group 15) or 25 (group 25) mg/kg/day of SAD.

Mycotoxin Analysis

To verify that postnatal exposure of offspring to SAD occurred following exposure to dams' milk, SAD levels of gastric contents and prosencephalon of pups were analyzed by high performance liquid chromatography (Series 10LC, LC-95 spectrophotometric detector, Perkin-Elmer, Norwalk, CT) using ultraviolet detection (26). Tissue samples were homogenized in distilled water, treated with 1 N hydrochloric acid (Fisher Scientific, Fairlawn, NJ) and acetonitrile (Burdick and Jackson, Muskegon, MI), centrifuged and extracted twice with hexane (Burdick and Jackson). Following addition of ethyl acetate, the solution was evaporated to dryness and the residue reconstituted in solvent (mobile phase) consisting of a 5:3:0.5:0.5 mixture of acetonitrile-water-glacial acetic acid-tetrahydrofuran. Samples were chromatographed within 12 hours on a reverse phase C_{18} (10 μm) column (Rainin Instrument Co., Woburn, MA). The flow rate of the mobile phase was 1.5 ml/min across a UV absorbance cell set at 339 nm with sensitivity of 0.5 absorbance units full scale. Standard recovery of 50 μl injections approached 90%.

Maturational and Behavioral Measures

The tests used here and summarized below were essentially standard methods described for use in mice and rats (1, 2, 13).

Body weight. Each pup was weighed on PND1, 3, 5, 7, 9, 11, 13, 15, 18, 20, 22, 25, 28, 31, and 34.

Incisor eruption. All pups were observed daily from PND7 until both upper and lower incisors had erupted.

Eye opening. All pups were observed daily from PND12 until eyelids of both eyes were completely separated.

Testes descent. Male pups were observed daily from PND21 until both testes descended.

Vaginal opening. Female pups were observed daily from PND30 until the vagina opened.

Surface righting reflex. Each pup was given two successive trials/day (between 6:00 to 6:20 a.m.) from PND1 to 4 and timed from being placed in a supine position until it had righted itself on all four feet in ≤2 seconds. Surface righting reflects the development of labyrinthine and body righting mechanisms (2,13).

Negative geotaxis. This behavior was observed daily (between 6:40 to 7:15 a.m.) on PND5 to 7 at an angle of 25°. Pups were timed for completing a 180° turn when placed in a head down position on an inclined surface. Maximum time allowed was 60 seconds. Negative geotaxis reflects development of vestibular function and associated integrated motor responses needed for space orientation (2).

Olfactory discrimination. This test was conducted on PND8, 10, 14 and 16 between 7:15 to 8:15 a.m. The apparatus consisted of a plastic container (35 × 13 × 12 cm), two small bins, a wire screen suspended 7 cm over the bins, and a clear plastic cover placed over the container. A bin filled with soiled "home" bedding was placed at one end of the container with the second bin, filled with clean bedding, at the opposite end. A line was drawn on the screen above each bin. Each pup was placed on a centrally located 3 cm square marked on the screen, and the latent time for crossing the line above the "home" bedding with head plus both forepaws was timed. Maximum time allowed was 2 minutes. Central placement of the pup was balanced by facing the pup toward or away from the experimenter on alternating test days. Age of home bedding averaged 2 to 4 days. This test reflects a nest seeking response mediated by the olfactory system (15).

Hindlimb grip. This test was conducted on PND14, 17 and 20 between 7:15 to 8:15 a.m. A steel wire (20 cm long by 0.2 cm thick) was supported between two poles. Each pup was gripped at the base of its tail and suspended above the wire until it grasped the wire with both forepaws. The pup was lowered, and when released pulled up and grasped the wire with all four paws. Maximum time allowed for this synergistic hindlimb support, the dependent

TABLE 1

BODY WEIGHT GAIN OF PREGNANT AND NURSING DAMS GAVAGED WITH VEHICLE (DMSO) OR SECALONIC ACID D (SAD) ON GESTATIONAL DAY (GD) 13 OR POSTGESTATIONAL DAYS (PGD) 1 TO 10

	Treatment		No. of	Mean Pregnancy Body Weight (g)			Mean Nursing Body Weight (g)		
Group†	GD	PGD	Mice	GD 7	GD 12	GD 18	PGD 1	PGD 10	PGD 21
N	DMSO	DMSO	10	37.1 ± 0.6	46.1 ± 1.9	50.4 ± 2.6	37.5 ± 1.2	41.6 ± 2.0	37.7 ± 1.4
15	DMSO	SAD	11	—*	—*	—*	36.9 ± 1.1	40.6 ± 1.9	36.9 ± 1.4
25	DMSO	SAD	10	37.4 ± 0.8	43.3 ± 0.5	60.7 ± 1.5	39.2 ± 2.1	42.1 ± 1.1	39.1 ± 1.3
P	SAD	DMSO	8	36.7 ± 0.9	44.3 ± 0.5	55.8 ± 1.0	41.2 ± 1.3‡	47.1 ± 2.2	44.9 ± 1.5‡

Values are means ± SEM.

*Measurements not taken for analysis.

†N, 15, and 25 = 0, 15 and 25 mg/kg SAD (postnatal exposure) and P = Prenatal exposure to 25 mg/kg SAD (positive behavioral teratogenic control).

‡Significantly different from corresponding N (negative control) values, $p<0.05$.

DMSO = dimethylsulfoxide vehicle.

variable, was 15 seconds. Hindlimb grip measures motor coordination requiring synergistic limb activity as well as muscle strength (2).

Statistical Analyses

Maternal weight gains, lengths of gestation, offspring sex ratio, progeny birth weight, maturational parameters and negative geotaxis response were analyzed by repeated measure analysis of variance (ANOVA) followed by the least significant difference (LSD) test. Treatment effects on offspring weight gains and olfactory discrimination were analyzed by split plot ANOVA. Surface righting reflex and hindlimb grip were analyzed by the Chi-square test. The data for individuals in each litter were averaged when appropriate, and the litter was used as the experimental unit for all tests with postnatal treatment considered as between group variable. The accepted level of significance was $p \leq 0.05$.

RESULTS

Maternal Weight Gain

Pregnancy body weights of dams in groups N, 25 and P were comparable at the beginning of SAD treatment (GD13) and did not significantly differ during the remaining gestation period (Table 1). Animals in group 15 were not weighed during gestation. Nursing dams treated with 25 mg/kg of SAD during gestation (positive behavioral teratogenic control, group P) weighed significantly more than dams from other treatment groups on PGD1, $F(3,36) = 2.30$, $p<0.05$, and PGD 21, $F(3,36) = 5.79$, $p<0.05$ (Table 1). Compared to negative control group N, the gains in weight were 10% and 19% respectively on PGD1 and 21. No other physical or behavioral changes were seen in dams exposed prenatally or postnatally to SAD.

Gestational Birth Records

Length of gestation, sex ratio at birth within litters, numbers of live or dead offspring delivered and body weights of female offspring were not significantly affected by prenatal treatment (Table 2). However, on PND1, birth weights of male offspring of group P dams were significantly reduced by 5% of the negative control group, $F(3,22) = 3.22$, $p<0.05$ (Table 2).

Mycotoxin Levels in Offspring

SAD residues were not detected in the gastric contents from neonates (PND1) in any group (data not shown). Significant levels

TABLE 2

GESTATIONAL AND BIRTH RECORD OF PREGNANT MICE GAVAGED WITH VEHICLE (DMSO) OR SECALONIC ACID D (SAD) ON GESTATIONAL DAY (GD) 13 OR POSTGESTATIONAL DAYS (PGD) 1 TO 10

	Treatment		No. of	Length of Gestation	No. of Live Pups/Litter	M:F Ratio	Total No. Dead Pups	PND1 Weight (g)	
Group†	GD	PGD	Litters*	(days)	on PND 1	(litter)	on PND 1	M	F
N	DMSO	DMSO	10	21.0 ± 0.3	11.1 ± 0.7	1.6 ± 0.4	1	1.68 ± 0.1	1.61 ± 0.1
15	DMSO	SAD	11	20.9 ± 0.1	12.9 ± 0.7	1.2 ± 0.2	1	1.67 ± 0.1	1.56 ± 0.1
25	DMSO	SAD	10	21.0 ± 0.2	12.0 ± 0.7	1.0 ± 0.1	2	1.64 ± 0.1	1.57 ± 0.1
P	SAD	DMSO	8	21.1 ± 0.3	12.5 ± 0.8	1.5 ± 0.5	1	1.59 ± 0.2‡	1.57 ± 0.3

*Number of litters also represents number of successful pregnancies.

Values are means ± SEM.

†N, 15, and 25 = 0, 15 and 25 mg/kg SAD (postnatal exposure) and P = Prenatal exposure to 25 mg/kg SAD (positive behavioral teratogenic control).

‡Significantly different from corresponding N (negative control) values, $p<0.05$.

M = Male, F = Female, PND1 = Postnatal Day 1, DMSO = dimethylsulfoxide vehicle.

TABLE 3

SECALONIC ACID D (SAD) LEVELS ON POSTNATAL DAY (PND) 10 IN THE PROSENCEPHALON AND GASTRIC CONTENTS OF SUCKLING MICE EXPOSED TO VEHICLE (DMSO) OR SECALONIC ACID D (SAD) ON GESTATIONAL DAY (GD) 13 OR POSTNATAL DAYS 1 TO 10

	Treatment		SAD Prosencephalon (ng/g)		SAD Gastric Contents (ng/g)	
Group*	GD	PGD	Male	Female	Male	Female
N	DMSO	DMSO	0.0	0.0	0.0	0.0
15	DMSO	SAD	0.0	0.0	125.9 ± 12.9‡	91.5 ± 7.3‡
25	DMSO	SAD	0.0	0.0	149.9 ± 39.9‡	423.4 ± 12.2‡
P	SAD	DMSO	0.0	0.0	0.0	0.0

Values are means ± SEM for 4 to 7 mice/sex/group.

*N, 15, and 25 = 0, 15 and 25 mg/kg SAD (postnatal exposure) and P = Prenatal exposure to 25 mg/kg SAD (postive behavioral teratogenic control).

PGD = postgestational day; DMSO = dimethylsulfoxide vehicle.

‡Significantly different from corresponding negative control (N) values, $p<0.05$.

of mycotoxin were detected on PND10 only in gastric contents of male, $F(3,15) = 132.7$, $p<0.0001$, and female, $F(3,16) = 799.48$, $p<0.0001$ pups (groups 15 and 25) which had suckled postgestationally SAD-exposed dams (Table 3). A dose-dependent accumulation of SAD was observed. Detectable SAD residues were likewise absent in brain tissue of all neonates (data not shown) and of PND10 pups of either sex from any treatment group (Table 3).

Postnatal Growth and Maturation

Offspring body weight gains of the negative and positive control groups (N,P) were comparable (Fig. 1). In contrast, pups of either sex exposed postnatally to SAD (groups 15 and 25) gained significantly less weight than their negative control groups, $F(14,980) = 66.41$, $p<0.0001$ (Fig. 1). From PND 18 to 34 the percentage body weight reductions ranged from 8% to 17% in both sexes of group 15. For pups of both sexes in group 25, the weight reductions from PND 15–34 ranged from 7% to 18%. As offspring matured in all groups, males within the group gained more weight than their female litter mates from PND 28–34, $F(1,980) = 22.75$, $p<0.0001$ (Fig. 1).

Compared to negative controls, male and female pups from both postnatal treatment groups 15 and 25 manifested significantly delayed times of incisor eruption, $F(3,70) = 10.90$, $p<0.05$ (Table 4). Testicular descent times in male pups from all SAD treatment groups (15, 25, P) were significantly delayed, $F(3,35) = 5.27$, $p<0.05$ (Table 4). In contrast, times of eye opening in pups of both sexes and times of vaginal opening in female pups were not significantly affected by treatment (Table 4).

Postnatal Behavior

Surface righting reflex. Since no significant sex-related difference in righting response was observed, the data were pooled for analysis. As shown in Fig. 2, the ontogeny of the surface righting was significantly delayed in all treatment groups on PND2, $\chi^2(3,78) = 11.54$, $p<0.05$, PND3, $\chi^2(6,78) = 19.17$, $p<0.05$, and PND4, $\chi^2(6,78) = 39.47$, $p<0.05$.

Negative geotaxis. The ontogeny of negative geotaxis was delayed in pups of both sexes in group 25 on PND7, $F(3,140) = 3.19$, $p<0.05$ (Fig. 3). Turning times in male and female pups from group 25 were 98% and 164% longer than pups in negative control groups, respectively. In addition, response times in male pups from group P were significantly delayed by 135% on PND 7. Female pups in group P and pups of both sexes in group 15 displayed nonsignificant delays in turning times.

Olfactory discrimination. The ontogeny of olfactory discrimination was significantly delayed in all pups exposed to SAD, $F(3,101) = 8.07$, $p<0.05$ (Fig. 4). Attraction to home cage bedding was significantly reduced in male and female offspring from groups 25 and P on all test days (PND 8, 10, 14, 16) and from

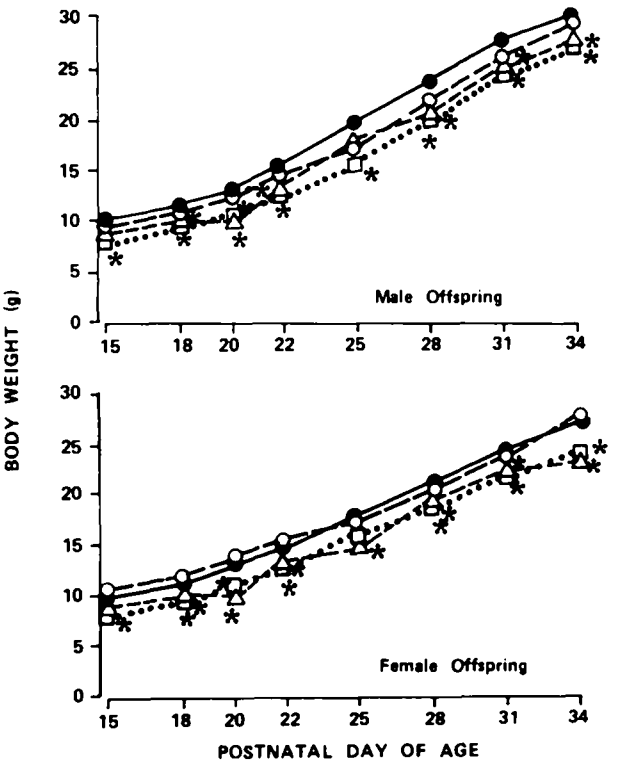

FIG. 1. Postnatal body weights of male and female mice exposed to vehicle (● = Negative Control) or secalonic acid D (SAD) on gestational day 13 (○ = Positive Control) or postnatal days 1 to 10 (△ = 15 mg/kg/day; □ = 25 mg/kg/day). Values are means for 8 to 11 litters/sex/group. *Significantly different from negative control values, $p<0.05$. Males are significantly different from female litter mates from PND28–34, $p<0.05$.

TABLE 4

APPEARANCE OF PHYSICAL LANDMARKS OF MATURATION IN SUCKLING MICE EXPOSED TO VEHICLE (DMSO) OR SECALONIC ACID D (SAD) ON GESTATIONAL DAY (GD) 13 OR POSTNATAL DAYS (PND) 1 TO 10

Group*	Treatment GD	Treatment PGD	PND of Incisor Eruption Male	PND of Incisor Eruption Female	PND of Eye Opening Male	PND of Eye Opening Female	PND of Testes Descent	PND of Vaginal Opening
N	DMSO	DMSO	9.2 ± 0.1	9.1 ± 0.1	14.5 ± 0.2	14.7 ± 0.2	22.0 ± 0.2	31.5 ± 0.2
15	DMSO	SAD	10.1 ± 0.1‡	10.1 ± 0.1‡	14.7 ± 0.2	14.9 ± 0.2	22.7 ± 0.2‡	31.7 ± 0.3
25	DMSO	SAD	9.6 ± 0.1‡	10.8 ± 0.2‡	14.9 ± 0.2	14.9 ± 0.2	22.8 ± 0.2‡	31.8 ± 0.2
P	SAD	DMSO	9.3 ± 0.1	9.3 ± 0.1	14.6 ± 0.3	14.6 ± 0.3	23.1 ± 0.2‡	31.6 ± 0.2

*N, 15, and 25 = 0, 15 and 25 mg/kg SAD (postnatal exposure) and P = Prenatal exposure to 25 mg/kg SAD (positive behavioral teratogenic control).

PGD = Postgestational day; DMSO = dimethylsulfoxide vehicle.

Values are means ± SEM for 8 to 11 litters/group.

‡Significantly different from corresponding N (negative control) values, $p<0.05$.

group 15 on PND 10 and 14 (Fig. 4). Postnatal SAD treatment produced a significant dose-response effect in both sexes.

Hindlimb grip. Since sex-related differences within groups were not observed, data were pooled for analysis. As seen in Fig. 5, the percentages of pups in all treatment groups gripping the wire synergistically with all paws were significantly reduced relative to negative control pups on PND 14, $\chi^2(6,78) = 15.78$, $p<0.05$, PND17, $\chi^2(6,78) = 43.61$, $p<0.05$, and PND 20, $\chi^2(6,78) = 31.42$, $p<0.05$.

DISCUSSION

The SAD doses used in this study were previously shown to be structurally teratogenic to rats (19) and mice (24) following multiple in utero exposures and behaviorally teratogenic to mice (29) following a single in utero exposure. The experimental design in this study, however, called for a positive behavioral teratogenic control group in order to establish concurrent test system sensitivity and to permit comparison of relative test system stability. Under the conditions of the present study, the prenatal dose of 25 mg/kg SAD on GD 13, which created positive control group P, produced developmental neurobehavioral deficits similar to those described in an earlier study (29) but did not otherwise adversely affect reproductive outcome (except for a 5% reduction in male neonatal body weight) or maternal health as measured by length of gestation, body weight gains and maternal behavior during pregnancy and nursing (Tables 1 and 2).

SAD-induced maturational effects were related to sex and time of SAD exposure. While only postnatal SAD exposure (groups 15 and 25) significantly delayed the incisor eruption time of both male and female pups, the prenatal and postnatal exposure levels, which produced no effect on the times of eye opening (in either sex) and female vaginal opening, significantly delayed testicular descent times in male pups (Table 4). It was unclear why SAD exposure selectively affected male neonatal body weights and testicular descent times. However, in utero exposure to certain drugs that are not sex hormones can produce decreased concentrations of testosterone and profound demasculinization in the

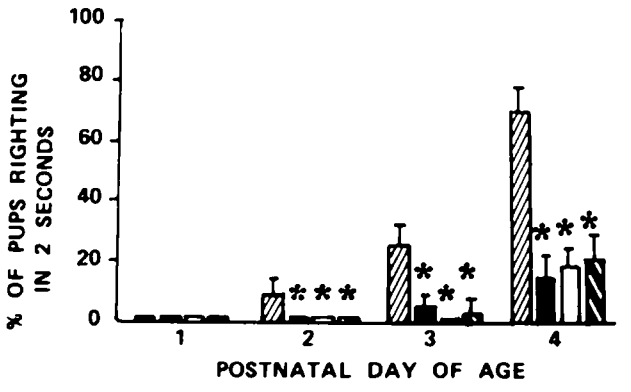

FIG. 2. Surface righting reflex response of mice exposed to vehicle (lightly hatched bars = Negative Control) or secalonic acid D (SAD) on gestational day 13 (heavily hatched bars = Positive control) or postnatal days 1 to 10 (solid bars = 15 mg/kg/day; open bars = 25 mg/kg/day). Values are means ± SEM for 8 to 11 litters/group. *Significantly different from negative controls, $p<0.05$.

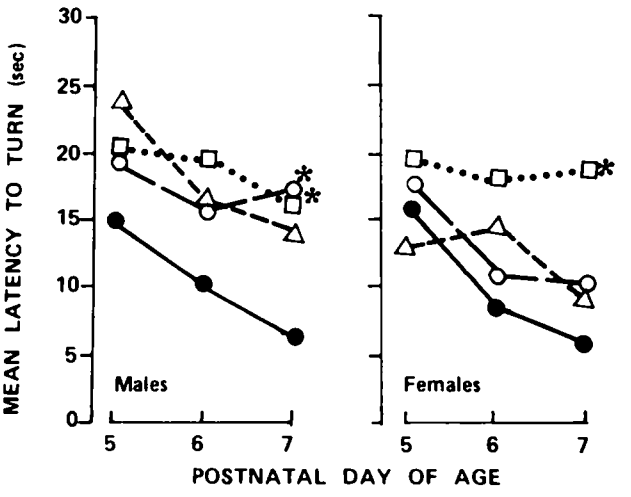

FIG. 3. Development of negative geotaxis (angle = 25°) in male and female mice exposed to vehicle (● = Negative Control) or secalonic acid D (SAD) on gestational day 13 (○ = Positive Control) or postnatal days 1 to 10 (△ = 15 mg/kg/day; □ = 25 mg/kg/day). Values are means for 8 to 11 litters/sex/group. *Significantly different from negative control values, $p<0.05$.

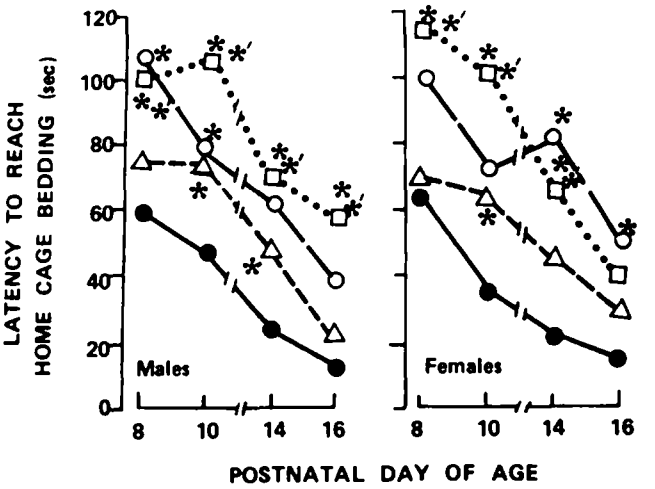

FIG. 4. Development of olfactory discrimination of male and female mice exposed to vehicle (● = Negative Control) or secalonic acid D (SAD) on gestational day 13 (○ = Positive Control) or postnatal days 1 to 10 (△ = 15 mg/kg/day; □ = 25 mg/kg/day). Values are means for 8 to 11 litters/sex/group. *Significantly different from negative controls, $p<0.05$. *'Group 25 are significantly different from group 15, $p<0.05$.

developing male pup that may persist into adulthood (3). Developmentally, 3',5'-cyclic adenosine monophosphate (cAMP) plays a central role in regulatory mechanism involving sex hormones, neurotransmitters, cellular growth and cellular differentiation (36). Testicular hormones play crucial roles in the transabdominal movement of the testis and its descent into the scrotum (37). Since SAD is known to decrease cAMP (11) and brain monoamine levels (5) in developing pups, it is intriguing to suggest that the SAD-induced delay in testicular descent is causally related to a perturbation in the cAMP testicular hormone system.

During late preweaning and postweaning periods, postnatal exposure to low and high doses of SAD significantly reduced the growth of male and female pups. Compared to control, the overall weight decrements for the low (group 15) and high (group 25) doses were 9% and 8% for males respectively, and 9% and 5% for females, respectively. It was of interest that the body weight gains of the negative and positive controls (group P) were comparable. In the absence of overt SAD-induced maternal toxicity and abnormal maternal rearing behavior, it seemed unlikely that reduced milk production by the dam or the manifestation of taste aversion by suckling pups was responsible for malnutrition and reduced growth of progeny. Daily visualization of the milk-distended stomachs (through the translucent abdominal wall) of pups from control and treatment groups indicated comparable milk consumption. Thus, the most tenable hypothesis was that preweaning growth retardation appeared to be a reliable indication of toxicity following lactational exposure to SAD.

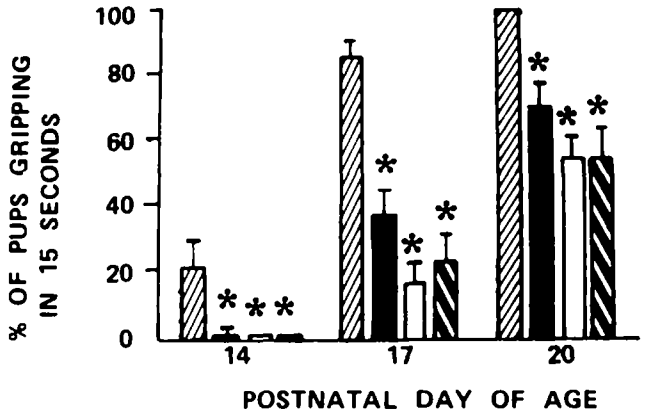

FIG. 5. Development of hindlimb grip reflex in male and female mice exposed to vehicle (lightly hatched bars = Negative Control) or secalonic acid D (SAD) on gestational day 13 (heavily hatched bars = Positive Control) or postnatal days 1 to 10 (solid bars = 15 mg/kg/day; open bars = 25 mg/kg/day). Values are means ± SEM for 8 to 11 litters/sex/group. *Significantly different from negative control, $p<0.05$.

Based upon previously reported fetotoxic and gross teratogenic effects following in utero exposure, it seemed likely that transplacental absorption and distribution of SAD to the fetus were significant (19, 24, 29, 30). Recent data have confirmed the placental transfer of SAD in mice following the intraperitoneal administration of 30 mg/kg of C^{14} SAD in DMSO to pregnant mice (12). However, because of wide tissue distribution and low blood levels in the dam, only low SAD levels can cross the placental barrier (0.14% to 0.28% of total dose) and enter the fetal head (0.028% to 0.017% of total dose) 24 to 48 hours after dosing (12). The presence of behavioral teratogenic effects in the positive control pups in this study suggested that SAD crossed both the placental and the immature fetal blood-brain barriers. It seemed unlikely however, that postnatal transmission of SAD via the dams' milk occurred in the positive control neonates since no SAD was detected in the gastric contents. Based on a plasma elimination half-life of 20.5 hr (25), 0.097% was the theoretical amount present in the dams' body on postgestational day 1 after dosage on gestational day 13. Since all SAD-exposed offspring manifested subtle neurobehavioral and limited developmental effects, it was of interest that SAD was absent in the brain (prosencephalon) at a detectable level in both the prenatally and postnatally exposed pups, particularly the latter pups (groups 15 and 25) in which nanogram amounts of SAD were detected in the gastric contents. It seemed unlikely that ingested SAD was not absorbed from the pups' guts since neonatal rats given oral doses of SAD are known to manifest acute systemic toxicity (22,24).

A most important feature of this study was that prenatal and early postnatal exposure of developing mice to SAD at doses which did not produce gross birth defects in either sex or detectable brain SAD levels markedly altered neurobehavioral performance during the preweaning period. Some behavioral deficits were manifested prior to the detection of adverse treatment effects on growth and on maturation. The delays in the ontogeny of surface righting reflex, olfactory discrimination and hindlimb grip behaviors in pups of both sexes from all SAD-treated groups as well as the delayed responses to early preweaning tests in negative geotaxis in male pups exposed prenatally and pups in both sexes from the 25 mg/kg postnatal treatment group were indicative of developmental deficits in the functioning of the vestibular and body righting mechanisms, olfactory system, reflex coordination and/or the integration of neuromuscular activity. Although behavioral effects without detectable brain SAD levels may imply a non-CNS effect, the fact that brain monoamine levels of pups exposed to SAD are decreased in a similar experimental protocol is indicative of a central neurotoxic effect of SAD (5). This does not, however, preclude the possibility of other nonneural effects of SAD (20,24).

The neurochemical mechanisms involved in SAD-induced behavioral abnormalities have not been established. It has been suggested that dopaminergic (6,21) and serotonergic (14,34) mechanisms play important roles as antagonistic systems in the ontogeny of normal locomotor and arousal responses. Recent studies by Bolon and St. Omer utilizing an identical exposure

design indicate that prenatal (25 mg/kg) and early postnatal (15 and 25 mg/kg) exposure to SAD reduce brain levels of dopamine (DA) and norepinephrine (NE) in preweaned offspring by 7% to 74%; greater neurotransmitter decreases follow exposure to the higher SAD doses (5,30). Similarly, serotonin (5-HT) and 5-hydroxyindoleacetic acid (a serotonin metabolite) levels are reduced by 30% to 71% (5). The effects on delaying the ontogeny of neurochemistry are significant at all SAD dose exposures except for NE and DA levels in the low postnatal exposure pups (5). The demonstrated preference of normal rodent pups for home nest material is a response mediated by the olfactory system (15). Several studies suggest a modulatory role of catecholamines in rodent olfactory function (9) which can be suppressed by neonatal treatment with the catecholaminergic neurotoxin 6-hydroxydopamine (15). It is of interest that SAD doses which attenuated pup preference for home nest odor in this study, also reportedly reduce NE and DA levels in the prosencephalon (which included the olfactory cortex) (5). A physiologic basis for SAD-induced neurotoxicity has also not been established. It has been suggested that SAD uncouples cellular energy synthesis which then disrupts cell growth and organogenesis, including neural development during prenatal and early postnatal periods of development (16,17). Extensive neurogenesis, synaptogenesis, myelination and neurochemical synthesis occur in the developing brain both during organogenesis (4, 16, 27, 33) and during postnatal development, up to PND30 in mice and for several months in human infants (4,27). In our study SAD exposure during prenatal or early postnatal critical periods of neural development led to subtle neurochemical deficits (5) which were manifested as neurobehavioral changes even in the absence of detectable neurotoxin brain levels or gross morphologic CNS alterations.

In this study, the behavioral teratogenicity of SAD was unequivocal following both prenatal and early postnatal exposure. Nursing mothers exposed to environmental neurotoxins may, therefore, place their suckling offspring at risk.

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