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Prenatal Effects of Alcohol on Adult Learning in Rats

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ABEL, E. L. *Prenatal Effects of Alcohol on Adult Learning in Rats*. PHARMAC. BIOCHEM. BEHAV. **10**(2) 239–243, 1979.—In an initial study, the rate of blood alcohol disappearance was not significantly different in pregnant compared to nonpregnant rats, but blood alcohol levels were significantly different depending on dose. In a second study, pregnant rats received daily administrations (p.o.) of ethanol (30% w/v) in single doses throughout gestation. Pair-fed vehicle-treated, and nondrug-treated rats fed ad lib served as controls. All pups were removed from their biological mothers at birth and were raised by nondrug-treated surrogate mothers. At five months of age, both male and female offspring prenatally exposed to ethanol weighed less than controls and female offspring performed significantly worse than the offspring of vehicle-injected pair-fed control mothers, on a two-way shock-avoidance task. There were no significant group differences, however, for either sex in water-escape maze learning.

Alcohol Pregnancy Growth Learning/Memory

IN 1973, Jones and Smith [7] reported that the offspring of chronic alcoholic women were characterized by a pattern of anomalies consisting of pre- and postnatal growth deficiencies, physical malformations, and mental retardation, which they called the "fetal alcohol syndrome." Since that inital report, many additional cases of the fetal alcohol syndrome have been observed, and a recent review of this disorder currently places its incidence at one-to-two live births per 1,000 and partial expression of the syndrome at three-to-five per 1,000 [5].

Although the physical anomalies observed in conjunction with the fetal alcohol syndrome appear to be due to the direct effects of alcohol, the clinical literature does not permit the elimination of conditions secondary to alcohol intake, e.g., altered nutrition, as etiological factors in the growth retardation and impairment of cognitive function associated with this disorder. Since these issues are difficult to resolve on the basis of clinical studies, various "animal models" have been employed to examine such issues under controlled laboratory conditions.

EXPERIMENT 1

Peak blood alcohol levels and the rate of blood alcohol disappearance were determined in pregnant rats intubated with different doses of alcohol. In a prior study (manuscript in submission), using doses of 1 and 2 g/kg of ethanol, pregnancy did not affect rate of blood alcohol disappearance, corroborating previous studies [8]. However, peak blood alcohol levels were observed to be significantly higher in nonpregnant rats compared with pregnant females. In the present study the effects of higher doses of ethanol, comparable to those used in subsequent behavioral studies (see below), were examined vis à vis blood alcohol levels and rate of blood alcohol disappearance.

METHOD

Fifteen-to-20 day timed-pregnant and nonpregnant Long-Evans hooded rats (75–85 days of age) were injected (PO) with 2, 4, or 6 g/kg of ethanol (30% w/v). A minimum of seven animals per group were tested. Beginning at 0.5 min after intubation, blood samples (25 μl) were taken from the tip of the tail at 0.5, 1.25, and at hourly intervals from 2-20 hr (except for 10–14 hr for the 6 g/kg dose), depending on the dosage administered. Animals given the 6 g/kg dose were divided into two subgroups. The first subgroup was intubated in the a.m. and blood samples were taken as just described. The second subgroup was intubated in the p.m. and blood samples were taken beginning in the a.m. at 14 hr after drug administration. Blood was deproteinized with $ZnSO_4$ and Ba $(OH)_2$ following which propanol (25 μl) was added as an internal standard. Blood samples were then refrigerated until they could be analyzed by gas chromatography according to the method of Greizerstein and Smith [6]. Group differences were evaluated by means of analysis of variance for repeated measures and by "t" tests.

RESULTS AND DISCUSSION

The data are presented in Fig. 1. Following administration of the 2 g/kg dose of alcohol, there were no significant differences in overall blood alcohol levels although the peak blood alcohol levels in the pregnant and nonpregnant animals were significantly different (69.6 ± 5.6 and 98.9 ± 8.7 mg% respectively; $t=1.96$, $df=22$, $p<0.05$, one-tailed), corroborating our previous study (manuscript in submission) showing a higher peak blood alcohol concentration in nonpregnant animals at this dose. However, the rate of blood alcohol disappearance, as determined by analysis of slopes calculated from the regression curves for each animal from the

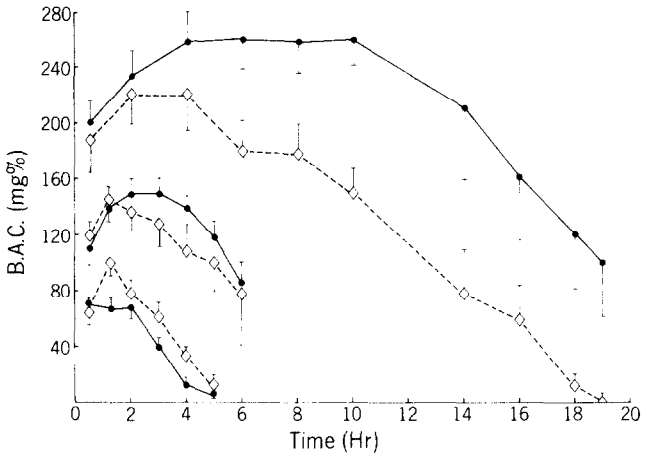

FIG. 1. Blood alcohol levels (mean ± SEM) in pregnant (●——●) and nonpregnant (◇- - - -◇) female rats following oral administration of alcohol (30% w/v). Lower curves: 2 g/kg; intermediate curves: 4 g/kg; upper curves: 6 g/kg (not all points are included). A minimum of seven animals are represented by each curve.

point of peak blood alcohol concentration, were not statistically significant.

Following administration of the 4 g/kg dose of alcohol, peak blood alcohol levels were not reached until 2-3 hr after drug administration in the pregnant animals compared to approximately 1–2 hr for the nonpregnant females. Overall blood alcohol levels in the pregnant animals were slightly (but not statistically significant) higher in the pregnant animals compared to nonpregnant females. The groups×time interaction was also not statistically significant. Peak blood alcohol level in the pregnant group was 150.6 ± 10.2 mg% compared to 142.0 ± 12.4 mg% for nonpregnant females. Differences in rate of blood alcohol disappearance between the two groups, were not statistically significant.

Following administration of 6 g/kg of alcohol, overall blood alcohol levels in animals intubated in the a.m. were higher in the pregnant animals, $F(1,18)=6.44$, $p<0.02$. The groups×time interaction was also significant, $F(16,288)=3.10$, $p<0.01$. However, the peak blood alchohol level for the pregnant animals (262.4 ± 19.3 mg<) was not statistically higher than that for the nonpregnant females (222.8 ± 27.3 mg%). Peak blood alcohol levels were reached approximately 5 hr after drug administration in the pregnant group compared to approximately 3 hr for the nonpregnant group. As can be seen from the Figure, blood alcohol levels remained at or near their peak concentration for approximately 6 hr in the pregnant group compared to only 2 hr for the nonpregnant group, and were still appreciably higher at 20 hr after drug administration. Group differences (sub-group 2) in rate of blood alcohol disappearance, calculated beginning at 14 hr after drug treatment, were not statistically significant.

These data demonstrate that following a low dose of alcohol, blood alcohol levels do not rise as high in pregnant rats as in nonpregnant females but at higher doses, this relationship is reversed and blood alcohol levels rise higher in pregnant females. Although an increased volume of distribution, associated with a pregnancy-related increase in total body water, could explain the results observed with the lower dose, some other explanation clearly must apply to the results of the higher dosage treatment. The present study, however, does not lend itself to any apparent explanation.

EXPERIMENT 2

Although growth retardation and various physical anomalies are the most dramatic characteristics of the fetal alcohol syndrome, impairment of cognitive function is now recognized as "the most serious defect and probably the most sensitive manifestation of maternal alcohol abuse" [10]. While impairments in adult learning in animals exposed to alcohol in utero have been previously reported [4,11], the failure to include controls for altered nutrition and/or postnatal maternal factors (e.g., [2,3], does not permit unequivocal conclusions to be drawn regarding the direct versus indirect effects of prenatal exposure to alcohol on cognitive development. In a previous study from this laboratory incorporating such controls, no significant impairments in learning were observed [2]. However, it is possible that the doses used in that experiment may have been below the threshold necessary for manifestation of such an effect. Accordingly, the present study investigated the effects of higher doses of ethanol than those previously tested, on body weight and learning/memory performance.

METHOD

One hundred and fifty timed pregnant nulliparious female Long-Evans hooded rats (Blue Spruce Farms), approximately 75–100 days of age and weighing approximately 220–250 g, were individually housed in Plexiglas cages and were maintained under relatively constant temperature (22°C) and humidity (30–40%) with 12 hr of light per day (7:00 a.m. to 7:00 p.m.).

On the day of their arrival from the supplier (Day 1 pregnancy), the animals were assigned to one of five subgroups: G 4—animals received 4.0 g/kg of ethanol daily; G 6—animals received 6.0 g/kg of ethanol daily; G 4P—animals were treated exactly as G 4 animals except for the isocaloric substitution of sucrose for ethanol intubation. These animals also served as "pair-fed" controls for G 4 animals in that the amount of food and water each G 4P animal received was conditional upon the food (Tecklad 4% mouse-rat diet) and water consumption of an animal with which it had been paired by body weight in group G 4; G 6P—animals were treated exactly like G 6 animals except for the isocaloric substitution of sucrose for ethanol intubation. These animals also served as "pair-fed" controls for G 6 animals; G C—animals did not receive any drug or vehicle treatment and had no restrictions as to food or water intake.

Drug treatments commenced the same day that animals were assigned to their respective groups. All drug treatments were given between 9:00 a.m. and 11:00 a.m. each day. Ethanol (U.S.P.) or isocaloric sucrose solution (in distilled water) were administered intragastrically. The concentration of the ethanol was 30% w/v.

Animals in all subgroups were weighed daily. Records were kept of changes in body weight and food and water consumption throughout gestation. Comparable records were kept for neonates including mortality during the first four weeks of life. (These data are reported elsewhere [3]).

In addition to these experimentally treated animals, an equal number of pregnant animals were designated as "surrogate" mothers. These animals were not disturbed until they gave birth.

As the gestation period neared completion, cages were inspected twice daily. As soon as the first births were observed, cages were inspected hourly except between midnight and 8:00 a.m. Pups were culled to four males and four females per litter wherever possible (no attempt was made to select specific individual animals in culling), and the litter of eight was assigned to a surrogate mother that had delivered within 24 hr of the animals in the experimentally treated groups. (The pups born to these surrogate mothers were removed within 24 hr of each assignment.)

Animals were weaned at 21 days of age, and two or three animals of the same sex from each litter were placed in Plexiglas cages. Food and water were available ad lib.

PROCEDURES

Adult Body Weight

At five months of age, prior to the study of shock-avoidance learning, all animals were weighed and weights were recorded and analyzed (long-term measurements of body weight associates exclusively with in utero exposure to alcohol have not previously been reported in animals or in man). Only one male and female from different litters were used for weighing. (All litter-mates were not weighed since most had previously been sacrificed for other experiments.)

Two-Way Shock Avoidance

The method and apparatus for two-way shock-avoidance learning were essentially like that reported previously [12]. Subjects (N=12/sex/group) were placed individually into a standard two-compartment automated shuttle box in which they could avoid or escape being shocked through the grid floor by jumping over a barrier dividing the two compartments of the apparatus. A 10 sec light and an auditory stimulus served as the compound conditioned stimulus (CS), and a 2.0 mA constant current electric shock served as the unconditioned stimulus (UCS). If an avoidance response was not made during this period, the shock was presented and remained on until the animal jumped over the barrier. Each presentation of the CS and the performance of a jumping response constituted a single trial. The intertrial interval was 20 sec. Fifty such trials were given over a five-day period. In addition to determining the number of avoidance responses made, records were also kept of the time required to jump following shock presentation (escape latency) and the number of between-trial jumps not associated with the CS (general motor activity).

Water Maze Escape Learning

Animals (N=6/sex/group) were tested for their ability to learn the route of escape from a water-filled multiple (6-unit) T-maze similar to that described by Biel (1940). Water temperature was maintained at 22 ± 1°C.

The method used to test animals was similar to that described by Abel [2]. Animals were given five trials/day for two days. The intertrial interval was 1 min. The following measures of maze performance were recorded: (1) number of trials before the first errorless trial, (2) number of errorless trials, and (3) percent transfer:

$$\frac{\text{correct trials (Day 2) - correct trials (Day 1)}}{\text{total trials}} \times 100.$$

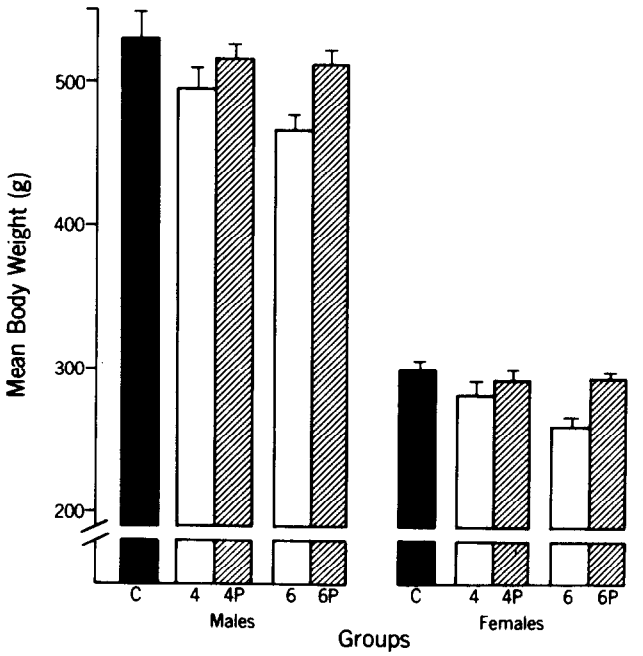

FIG. 2. Mean body weight (±SE) of rats prenatally exposed to 4 or 6 g/kg/day of ethanol (30% w/v), their pair-fed controls (4P, 6P), and ad lib controls (C), at five months of age.

No animal was allowed to remain in the maze for more than 4 min on any single trial so as to prevent exhaustion. Animals not escaping within 4 min on any single trial were guided to the escape ramp and were removed from the apparatus. These nonescaping trials were not included in the analysis. Additional trials were given for every nonescape trial.

RESULTS AND DISCUSSION

Body Weight

Body weights of adult offspring in the various groups are shown in Fig. 2. The effect of prenatal exposure to alcohol appeared to be dose-dependent with G 4 animals weighing less than ad lib and pair-fed controls and G 6 animals weighing least of all. Males and females were similarly affected, $F(4,55)=(3.71,5.23)$, $p<0.01$ for males and females respectively. These observations suggest that the growth retardation resulting from prenatal exposure to alcohol is not transient, especially when the level of exposure is high. The decreased body weights of Group 6 animals was not likely due to altered maternal nutrition during pregnancy since Group 6P did not differ markedly from the ad lib control group.

Two-Way Shock-Avoiance

Inadvertently, only one-half the males (N=6) in each of the five groups were tested. Group 6 males performed slightly better than 6P males whereas Group 4 males performed worse than its pair-fed controls. These differences were not significant.

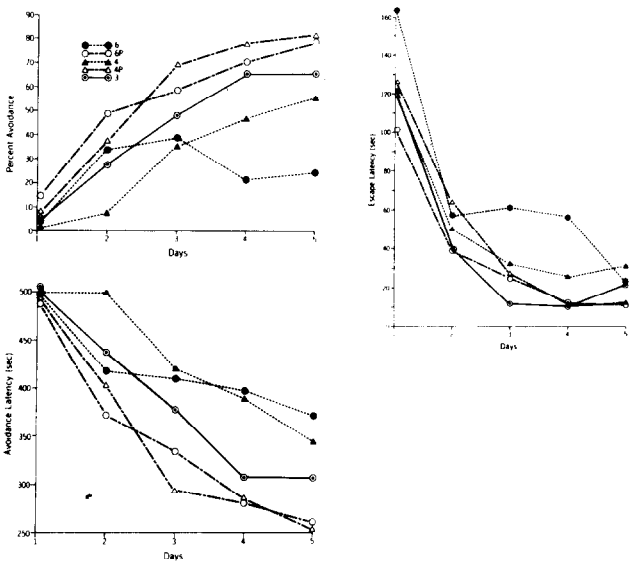

FIG. 3. Upper left figure: Mean percent avoidance (females) on two-way shock avoidance learning task during five consecutive test days (50 trials per day); Lower left: Mean avoidance latencies following onset of CS; Right: Mean escape latencies following onset of UCS. (N=12/group).

The data for the females with respect to number of avoidances, avoidance latency, and escape latency are presented in Fig. 3. The ad lib (G C) and two pair-fed control groups (G 4P, 6P) made slightly more avoidances on the first days of testing than did the two drug-treated groups (G 4, 6) and continued to improve as testing continued. Although G 4 animals did not perform as well as G 4P animals on the first three days of testing, they continued to improve on Days 4 and 5. Animals in Group 6, however, did not continue to improve in terms of avoidance responses after Day 3 and, in fact, performance declined as testing continued. Both the group differences in mean avoidances and the Group × Days interaction were statistically significant, $F(4,55)=2.89$, $p<0.01$, $F(16,220)=41.2$, respectively.

Avoidance latency scores of the alcohol-treated females were greater than those for control groups. Differences between the two alcohol-treated groups were minimal except on Day 2 when the latency for Group G 4 animals was approximately 75 sec greater than those for G 6. Both the group differences and the Group × Days interaction were statistically significant, $F(4,55)=2.78$, $F(16,220)=2.24$, $p<0.01$ respectively.

There were too few intertrial interval jump scores to warrant statistical analysis, indicating that group differences were not due to differences in general motor activity. Escape latencies were slightly but not significantly greater for the alcohol-treated animals relative to controls.

None of the differences in water-escape maze learning were significant.

These data demonstrate that prenatal exposure to alcohol had deleterious effects on learning during adulthood only in female rats and only on avoidance learning. The reason that female and not male two-way avoidance learning was affected is difficult to determine. Previous studies from this laboratory have also noted that the behavior of females is more affected than males by perinatal exposure to alcohol [1]. Although it is possible that the males were less sensitive to the effects of alcohol in utero, the effects on body weight do not warrant such a conclusion since body weight was significantly reduced for both males and females.

GENERAL DISCUSSION

The data from Experiment 1 demonstrate that following a low dose of alcohol, blood alcohol levels do not rise as high in pregnant rats as in nonpregnant females but at higher doses, this relationship is reversed and blood alcohol levels rise higher in pregnant females. Although an increased volume of distribution, associated with a pregnancy-related increase in total body water, could explain the results observed with the lower dose, some other explanation clearly must apply in light of the results of the higher dosage treatment. These data are also not likely due to delayed absorption of alcohol from the gut due to the increase in food intake associated with pregnancy since absorption was not delayed in the pregnant animals relative to nonpregnant animals given the 2 g/kg. The present study, however, does not lend itself to any apparent explanation.

In addition to higher overall blood alcohol levels in pregnant animals, blood alcohol levels remained at peak concentration for almost 6 hr in the pregnant group and were still appreciably higher at 20 hr after drug administration. These observations are especially interesting in light of attempts to develop "animal models" for the "fetal alcohol syndrome," the purpose for which our studies were originally intended.

Many investigators have resorted to using liquid diets containing alcohol or have placed alcohol in the drinking fluid to achieve high levels of blood alcohol over a sustained period of time. A basic disadvantage of that procedure, however, is that animals differ widely in their consumption of these alcohol-containing solutions with the result that blood alcohol levels vary considerably among pregnant animals (and, therefore, among their fetuses as well). For example, a recent study by Martin and her co-workers [9] indicated that during the third trimester of pregnancy, consumption of ethanol in the form of liquid diet decreases considerably compared to the second trimester. Although speculative at this time, it is possible that this decrease is due to the prolonged elevation of blood alcohol levels in pregnant animals. These elevated levels could attenuate ad lib consumption either as a result of sedation or by affecting utilization and, therefore, requirement for further calories. By administering alcohol by gavage, we are able to administer the same amount of drug at the same time to each animal and are able to achieve relatively high blood alcohol levels over a sustained period of time, thus circumventing the problem of varying and fluctuating blood alcohol levels due to individual differences in amounts of ethanol consumed and differences in patterns of consumption. The disadvantage of this method, of course, is that intubation is somewhat stressful for animals. (Although pair-fed animals are equally stressed, it is possible that, relatively speaking, pair-fed animals are more stressed by the procedure since alcohol-treated animals may be slightly sedated at time of treatment.)

Alcohol exposure during both gestation and lactation produces long-term reductions in body weight in rats [10]. Long-term measurements of body weight associated exclusively with in utero expsure to alcohol, however, have not

hitherto been previously reported in animals or in man. Previous studies from this laboratory [2,3] showed that by seven days of age, rats prenatally exposed to 1, 2, or 4 g/kg/day of alcohol exhibited rapid "catch-up" growth whereas at 29 days of age those exposed to 6 g/kg/day were still significantly lighter in body weight than their pair-fed controls. The present study extends these observations and demonstrates that body weight remains retarded at five months of age for animals born to females exposed to 6 g/kg of ethanol throughout pregnancy.

Prenatal exposure to alcohol had deleterious effects on two-way shock-avoidance learning during adulthood in female rats. This finding corroborates previous reports of learning impairment resulting from prenatal ethanol exposure [4,11]. Previous studies, however, left open the possibility that the results were due to postnatal maternal factors rather than in utero exposure to ethanol. In the present study, animals were removed from their biological mothers shortly after birth so that these results cannot be attributed to an alcohol-related inhibition of postnatal maternal behavior. The absence of significant differences in water-escape learning suggests that some measures of learning/memory performance are not sensitive enough to reveal alcohol-induced deficits. Prenatal deficits in two-way shock-avoidance learning, but not Hebb-Williams maze learning, for example, have been reported by Bond and DiGiusto [4]. Conceivably, the deficits in learning may be specific and relate only to certain situations.

Although pair-feeding was included as a control procedure in the present study, there is still a possibility that these results were related to altered nutrition since alcohol may affect the transport of nutrients across membranes. Hence, although food intake was not different between alcohol-treated animals and their pair-fed controls, it is conceivable that the actual amount of nutrients available to the fetuses of the alcohol-treated mothers may have been markedly less than that available to the fetus of control animals. Whether availability is reduced to the point that fetal development is affected is not known.

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REFERENCES

1. Abel, E. L. Emotionality in offspring of rats fed alcohol while nursing. *J. Stud. Alcohol* **36:** 654–658, 1975.
2. Abel, E. L. Effects of ethanol on pregnant rats and their offspring. *Psychopharmacology* **57:** 5–11, 1978.
3. Abel, E. L. and B. A. Dintcheff. Effects of prenatal alcohol exposure on growth and development in rats. *J. Pharmac. exp. Ther.* In press.
4. Bond, N. W. and E. L. DiGiusto. Avoidance conditioning and Hebb-Williams maze performance in rats treated prenatally with alcohol. *Psychopharmacology* **58:** 69–71, 1978.
5. Clarren, S. K. and D. W. Smith. The fetal alcohol syndrome. *New Engl J. Med.* **298:** 1063–1067, 1978.
6. Greizerstein, H. B. and C. M. Smith. Development and loss of tolerance to ethanol in goldfish. *J. Pharmac. exp. Ther.* **187:** 391–399, 1973.
7. Jones, K. L. and D. W. Smith. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* **2:** 999–1001, 1973.
8. Kesaniemi, Y. A. Metabolism of ethanol and acetaldehyde in intact rats during pregnancy. *Biochem. Pharmac.* **23:** 1157–1162, 1974.
9. Martin, J. C., D. C. Martin, B. Radow and G. Sigman. Blood alcohol level and caloric intake in the gravid rat as a function of diurnal period, trimester, and vehicle. *Pharmac. Biochem. Behav.* **8:** 421–427, 1978.
10. Mulvihill, J. J. and J. J. Yeager. Fetal alcohol syndrome. *Teratology* **13:** 345–348, 1976.
11. Phillips, D. S. and G. L. Stainbrook. Effects of early alcohol exposure upon adult learning ability and taste preferences. *Physiol. Psychol.* **4:** 473–475, 1976.
12. Walters, G. C. and E. L. Abel. Effects of a marihuana homologue (Pyrahexyl) on avoidance learning in the gerbil. *J. Pharm. Pharmac.* **22:** 310–312, 1970.