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Effects of Alcohol Consumption During Pregnancy on Subsequent Maternal Behaviour in Rats

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HILL, L. G. J. AND L. W. MEANS. *Effects of alcohol consumption on maternal behaviour in rats.* PHARMAC. BIOCHEM. BEHAV. 17(1) 125–129, 1982.—Nine Holtzman rats were maintained from days 5–18 of pregnancy on a liquid diet of which ethanol constituted 35% of the calories while nine rats were pair-fed an isocaloric diet that had maltose-dextrin substituted for the ethanol. Beginning on day 19 of gestation and continuing for five days the dams in both groups were given a choice among lab chow, water, and their respective liquid diets. During the five days of choice, alcohol consumption dropped to approximately 20% of pre-choice levels. The dams and pups showed no behavioural withdrawal symptoms. While the alcohol-treated litters showed greater variability in litter size and more pup deaths during nursing, the maternal behaviour of the alcohol dams was essentially normal, with only their pup protectiveness being rated lower than that of the control dams.

Pregnancy and alcohol Alcohol and maternal behaviour Fetal alcohol model Alcohol withdrawal

VERY few studies have examined the effects of exposure to alcohol during pregnancy on the subsequent maternal behaviour of rodent dams. Abel [2] has shown that dams intubated with 2.0 g/kg/day of ethanol throughout pregnancy take longer to retrieve pups on the first day of nursing than do pair-fed controls. Also rat dams given 5% ethanol in their drinking water throughout pregnancy and lactation show slow retrieval responses [12], and mouse dams given 10% ethanol to drink throughout pregnancy and nursing show elevated exploratory behaviour on the first day of nursing. A recent study [5] that included only five ethanol dams failed to find a difference in pup retrieval latency between control animals and dams intubated twice daily with 4.0 g/kg ethanol from days 6–21 of pregnancy. Many investigators have observed increased cannibalism of pups following exposure to ethanol during pregnancy [3, 6, 21].

Several possible mechanisms could contribute to altered maternal behaviour following alcohol treatment during pregnancy. One, ethanol may affect lactation which could alter time in nest, mother-pup contact, and pup nutrition [1]. Bond [11] found that dams maintained on a liquid ethanol diet on days 1–17 of nursing, spent less time in the nest and engaged in more locomotor activity immediately following removal of the ethanol diet than did dams receiving a control diet. Two, the dam and pups may show altered behaviour on the first and second day postpartum due to withdrawal from alcohol, as it is well established that only 10–16 days continuous exposure to ethanol is necessary to induce withdrawal symptoms in rats [17,18]. Three, characteristics of the pups may elicit altered maternal behaviour by the dam. Mother-pup interactions during nursing have been shown to be affected by pup activity [25], smell, body temperature, pup size [8,9] and ultrasonic squeaks [15]. Pups that are small as a result of induced jaundice [13] or malnutrition [22] elicit slow retrieval and poor nest construction by mothers. Many studies have found that the offspring of dams treated with alcohol throughout pregnancy are lighter and smaller than the offspring of controls [4]. The decreased size is probably due to decreased consumption of ethanol containing fluids during pregnancy [24] and/or poor utilization of available nutrients [7,15].

To avoid some of the mother-pup interaction problems associated with giving dams alcohol during pregnancy, some investigators foster all or some of the pups to mothers that have been maintained on normal diets throughout pregnancy [2]. Of course, fostering does not eliminate withdrawal symptoms or altered characteristics of the pups which might elicit poor maternal care. Also, Beach and Jaynes [8,9] have shown that mother rats can discriminate their pups from aliens placed in the litter.

The present study was done to examine the effects of ethanol consumption during pregnancy on subsequent maternal behaviour under conditions that eliminate many of the possible mechanisms that can contribute to poor maternal behaviour. Thus, dams maintained on a liquid diet containing 35% ethanol derived calories on days 5–18 of gestation, but given a choice between the liquid diet, lab chow, and water on days 19–23, were compared to pair-fed control dams on

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maternal behaviour on days 1–20 of nursing. Giving the dams a dietary choice beginning shortly before birth eliminated the problems associated with sudden removal of alcohol for both the pups and the dams. Having dams raise their own pups eliminated problems associated with fostering. Pair-feeding the control dams was an effort to minimize the operation of mechanisms associated with nutritional differences.

METHOD

Subjects

Eighteen 120-day-old nulliparous female Holtzman rats were used as subjects in the experiment. Males of the same age and strain were used for studs. Throughout the experiment a 16-hour-light/8-hour-dark cycle was maintained with the lights being turned on at 7:00 a.m.

Breeding

A male was placed with groups of three or four females. Vaginal smears were examined daily until pregnancy was determined by the presence of sperm or continuous diestrous. Pregnant females were placed individually in standard wire mesh cages until five days prior to expected delivery. At this time they were moved to 46-cm × 23-cm × 20-cm plastic cages with stainless steel grid covers. The plastic cages contained wood shavings, and strips of paper towels were placed on the grid above the cage to be available for nesting material. Most of the cages were placed below mirrors suspended at a 45° angle which permitted nondisruptive observation of the dams and pups.

Diet Administration

On days 5–18 of pregnancy, nine dams were given free access to a liquid diet which contained 64 ml chocolate Nutrament (Mead Johnson, Inc.), 0.24 g Vitamin Diet Fortification Mixture (ICN Nutritional Biochemicals), 6.67 ml 95% ethanol (35% of total calories) for every 100 ml of diet. During this same period, nine dams were pair-fed an isocaloric diet that had 8.75 g maltose-dextrin substituted for the ethanol. Each dam in the control group was paired by weight with a dam in the alcohol group, and was given daily the amount of diet equal in volume and calories to that consumed by the alcohol-diet dam to which she was paired. Beginning on the nineteenth day of gestation, the alcohol dams were given free access to lab chow, water, and the liquid alcohol diet. The control dams were given free access to lab chow, water, and the appropriate amount of the liquid maltose diet. The dams were weighed every third day throughout pregnancy. On days 12 and 18 of gestation at approximately 9:00 a.m. which was one hour after the fresh diet was given, 25-μl blood samples were drawn from the tails of all the alcohol diet dams and three randomly selected maltose-diet dams to determine blood alcohol levels. Blood samples were taken from the maltose dams as both a check on the assay procedure and to determine if the blood-sampling procedure produced detectable stress-mediated changes in the pups. A gas chromatograph technique using l-propanol as the standard [10] was used to determine blood alcohol levels. Blood samples were tested within 4 hrs from the time of collection.

Observation

At birth, the performance of each dam in stripping the membranes, snipping the umbilical cord, ingesting the placenta, and licking the pups was observed. The observer, who spent the entire night in the laboratory making frequent checks on the dams and litters, also noted birth duration (time between the birth of the first and last pup), number of pups, number of pup deaths, activity of pups, and litter weight.

Beginning on the day after parturition, 30-minute observation periods were conducted at approximately 9:00 a.m. and 2:00 p.m. each day until the pups were 20 days old. A time sampling technique was used. Each dam was observed two minutes during the observation periods and her ongoing behaviour was recorded. The behaviours recorded included: grooming pups, nesting (nest construction), grooming self, eating, active (alert, but not engaged in maternal behaviour), and sleeping (lying down). The proportion of the litter nursing and whether the mother was in the same half of the cage as the majority of the litter (pup contact) during each 2 minute observation was also noted. At the end of each 30-minute observation period, each nest was rated on a 5-point scale according to the criteria established by Seitz [26]. Essentially, 0 corresponded to no nest, 1 a bare spot in the shavings for the pups, 2 a non-circular nest with no sides, 3 a circular nest with 2.5-cm high sides, and 4 a circular nest with 5.0-cm high sides. Then, the dams were tested for pup retrieval and protectiveness. The observer removed one pup from the nest, which was invariably at one end of the cage, and placed it at the opposite end of the cage. Retrieval time was the length of time between when the pup was released in the cage and the mother returned it to the nest. Pup protectiveness, was evaluated by assigning a rating, using a 5-point scale, to the dams reaction when the experimenter reached into the nest to procure a pup for the pup-retrieval test. A score of 0 indicated no reaction, 1 an alerting response, 2 an alerting and orienting toward the experimenter's hand, 3 an attempt to conceal the pups, and 4 an attempt to conceal the pups and an advance toward the experimenter's hand. The final behavioural test consisted of jangling keys over the cages to test for audio-induced seizures. The observer also recorded any alcohol withdrawal behaviours [15] that occurred during the first five observation days. All behavioural observations were made by the same observer.

RESULTS

Alcohol Consumption

The daily alcohol consumption of the experimental group is shown in Fig. 1. During the last two weeks of gestation, the dams consumed approximately 12 g/kg/day of ethanol. When a choice between the liquid alcohol diet, lab chow, and water diet was given, alcohol consumption dropped to approximately 20% of the original consumption. Blood alcohol levels determined on Days 12 and 18 were 92.6±11.5 mg/dl and 84.3±17.3 mg/dl respectively.

Gestation

The alcohol-diet and maltose-diet dams had gestation times of 21.67±0.25 and 22.11±0.41 days (Mean±S_{Mean}), respectively, a nonsignificant difference. The weight of each dam on day 5 of gestation was subtracted from her weight on day 15 to obtain an index of weight gain during pregnancy. The alcohol-diet dams gained 21.11±6.31 g while the maltose dams gained 24.22±3.88 g, a nonsignificant difference.

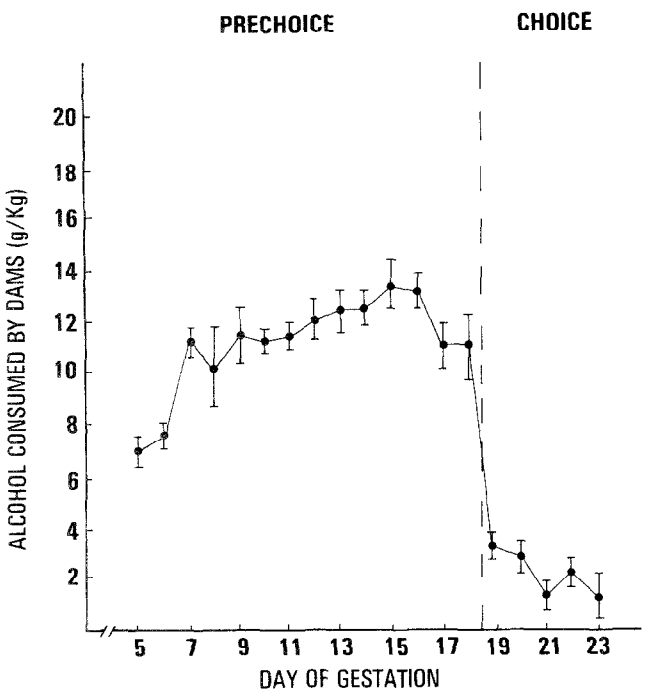

FIG. 1. Amount of alcohol consumed daily by alcohol dams expressed in g/kg. Vertical lines represent standard errors.

Litters

Table 1 summarizes measurements taken at the birth of the pups. The two groups did not differ on the mean number of pups per litter, proportion of males per litter, or mean pup weight (litter weight/litter size) at birth. The alcohol-diet litters were much more variable in number of pups, $F(8,8)=11.75$, $p<0.005$ and had significantly lower total weight, $t(16)=2.18$, $p<0.05$. The pups of eight of 9 maltose-diet litters were rated as being active at birth, while the pups of only 4 of 9 alcohol-diet litters were scored active, a nonsignificant difference. Also, the surviving alcohol and maltose pups did not differ in weight gain over the 20-day nursing period. However, during the 20-day nursing period, the alcohol mothers had 2.2 ± 0.6 pups die while the maltose mothers had 0.6 ± 0.2 pups die, a significant difference, $t(16)=2.51$, $p<0.05$. Finally, the litters of the 3 maltose-diet dams from whom blood samples were collected were indistinguishable from the other maltose-diet litters.

Alcohol Withdrawal Behaviour

No dam or pup was ever observed to have an audio-induced seizure or to display any other withdrawal behaviour during any of the 30-minute observation periods or during frequent informal observations made during the first few days after discontinuation of the alcohol diet.

Maternal Behaviour

Birth process. Birth duration data were collected on 7 alcohol-diet and 5 maltose-diet dams. Birth duration for the alcohol and maltose-diet dams were 109 ± 10 min and 91 ± 10 min (Mean$\pm S_{Mean}$), an insignificant difference. Also, the two

TABLE 1

NUMBER OF PUPS, PROPORTION OF MALES, LITTER WEIGHT, AND INDIVIDUAL PUP WEIGHT OF ALCOHOL- AND MALTOSE-DIET DAMS

Dependent Variable	Alcohol (n=9) Mean	S_{mean}	Maltose (n=9) Mean	S_{mean}	p
Number of Pups	10.0	1.1	11.3	0.3	n.s.
Proportion of Males	0.6	0.05	0.6	0.02	n.s.
Total Litter Weight	61.8 g	10.5 g	84.5 g	2.9 g	<0.05
Mean Pup Weight	6.8 g	0.3 g	7.7 g	0.4 g	n.s.

groups of mothers did not differ on licking of the pups, biting of the cord, ingestion of the membranes and placenta, or assumption of the nursing position in any discernable way.

Time-sampled behaviours. The mean frequency that the mothers were observed in pup contact, nesting activity, and pup grooming, and the mean proportion of pups observed to be nursing during the a.m. and p.m. observation sessions is presented in Fig. 2. An a.m. and a p.m. daily mean was determined for each block of 4 days. Each of these behaviours was examined with a three-way mixed factors analysis of variance (treatment × blocks of days × time of day). The treatment main effect was not significant on any of the behaviours. Both groups showed a significant decrease over blocks of days on pup contact, $F(4,64)=6.32$, $p<0.001$, pup grooming, $F(4,64)=2.96$, $p<0.05$, and nursing, $F(4,64)=5.49$, $p<0.001$. Also, both groups engaged in more pup contact, $F(1,64)=55.68$, $p<0.001$, and pup grooming, $F(1,64)=7.96$, $p<0.01$, during the morning than during the afternoon sessions.

Pup retrieval. The latency to retrieve pups (Fig. 2) was converted to logarithms and subjected to a three-way mixed factors anova (treatment × blocks of 2 days × time). Retrieval data taken after day 10 was discarded because most of the mothers in both groups were no longer retrieving their pups. The analysis revealed no significant main effects or interactions involving treatment.

Pup protection. Median ratings for protective behaviour are presented in Fig. 3. Chi-Square tests with Yates' correction performed on each block showed that the control group had significantly higher ratings on Days 1–4, $\chi^2(1)=6.25$, $p<0.02$, and on Days 5–8, $\chi^2(1)=5.63$, $p<0.02$. For the remainder of the experimental period, differences were non-significant. Friedman analysis of variance of protection ratings over blocks of days showed significantly different ratings in the alcohol group, $\chi^2(4)=14.60$, $p<0.01$, and in the control group, $\chi^2(4)=20.56$, $p<0.001$, over time. Observations of Fig. 3 reveals that both groups of dams became less protective over blocks of days.

Nest quality. The median rating within each 4-day block was determined for each dam in both groups (see Fig. 3). Chi-square analyses revealed no significant differences between groups during any block. Friedman analyses of median ratings over blocks showed a significant decline in ratings for the alcohol group, $\chi^2(4)=20.58$, $p<0.001$ and the maltose group, $\chi^2(4)=21.17$, $p<0.001$.

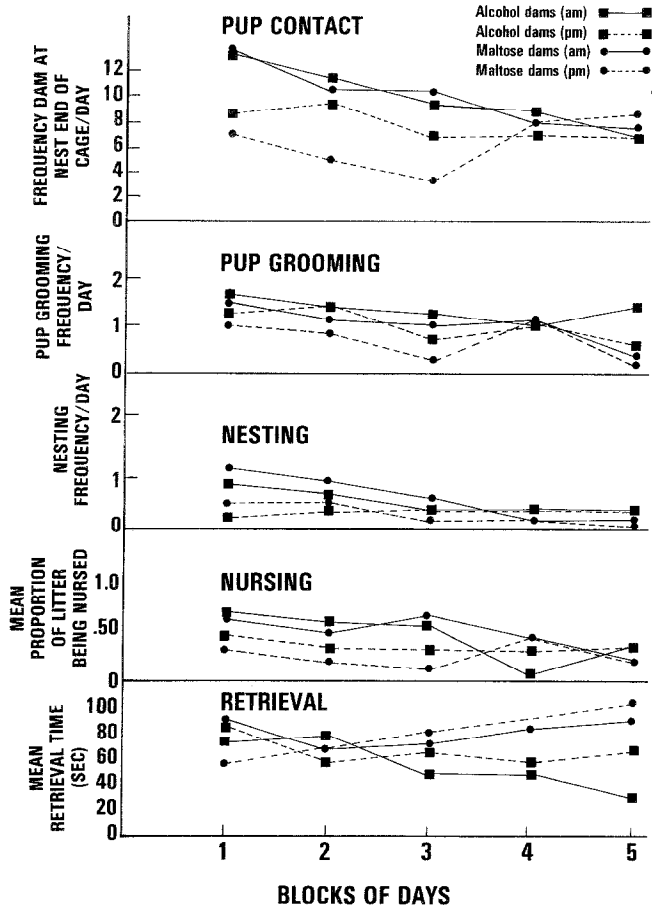

FIG. 2. Mean frequency of pup contact, grooming and nest building, mean proportion and pups nursing and mean retrieval latency for all dams throughout postpartum period. (All blocks represent four-day periods except for retrieval blocks, which represent two-day periods).

DISCUSSION

The overwhelming conclusion to be drawn from the present study is that when nutritional factors are controlled and potential withdrawal effects are eliminated, there is very little demonstrable difference in the maternal care of dams given a high dose of alcohol during pregnancy and that of control dams. The single significant difference between the alcohol- and maltose-diet dams was in the pup-protectiveness rating during the first eight days post-partum, with the maltose dams being rated more protective. However, even this difference is of little consequence in the laboratory setting where each litter is raised in a separate cage. There are essentially no stressful situations which would result in differential treatment of the pups by protective and non-protective mothers.

Differences in retrieval latencies reported by other investigators [2,12] may have been due to the fact that dams and pups were experiencing the effects of sudden cessation of alcohol [2], that the dams were still receiving alcohol [12], or that the alcohol pups were smaller than the controls [12,22]. In the present study, while the alcohol and maltose litters differed in total weight, there was no significant difference in mean pup weight. Also, the dietary choice procedure instituted during the last days of pregnancy and the first days of lactation eliminated the abrupt cessation of high doses of alcohol and any demonstrable behavioural withdrawal effects.

The observed increased pup mortality throughout nursing in the alcohol group is consistent with earlier reports [4]. The high pup mortality was probably due to some direct effect on the alcohol on the pups, as the maternal behaviour of the alcohol dams was essentially normal. Prenatal exposure to ethanol has been shown to disrupt normal immunity mechanisms [19], and produce many other physiological changes [4].

The blood alcohol levels observed in the present study are lower than those reported by other investigators [3,5] and could provide a possible explanation for our failure to find meaningful differences between the two groups. However, alcohol consumption of approximately 12 g/kg/day is quite high and no effort was made to draw samples at various times to determine peak blood alcohol levels. It has been shown that rats given continuous access to liquid ethanol diets obtain peak blood alcohol levels during the dark portion of the light cycle [18].

The present study clearly reveals that pregnant rats, when given the opportunity, will decrease their consumption of a liquid diet containing alcohol. This is consistent with the observation that pregnant women [20], monkeys [14], rats [22], and mice [24] all show decreased preference for alcohol. It is interesting to note that when given a choice, alcohol dams did not completely avoid alcohol ingestion, but consumed approximately 20% of their prechoice levels (Fig. 1). Possibly the pregnant rats were consuming just enough ethanol to avoid any of the unpleasantness of alcohol cessation.

Finally, the present experiment establishes an alternative model system for studying the effects of *in utero* exposure to alcohol on subsequent behavioural and physiological development. It provides a model that minimizes alcohol withdrawal-induced problems and eliminates the need for subjecting the pups to the potential stresses associated with fostering [8]. The authors feel that the method herein reported could be improved by pair feeding the dams on lab chow consumption during the 5 days of choice between the liquid diets, lab chow, and water. However, as the present study did not reveal a difference between the alcohol- and maltose-diet pups on mean weight at birth, pair feeding of lab chow may not be crucial.

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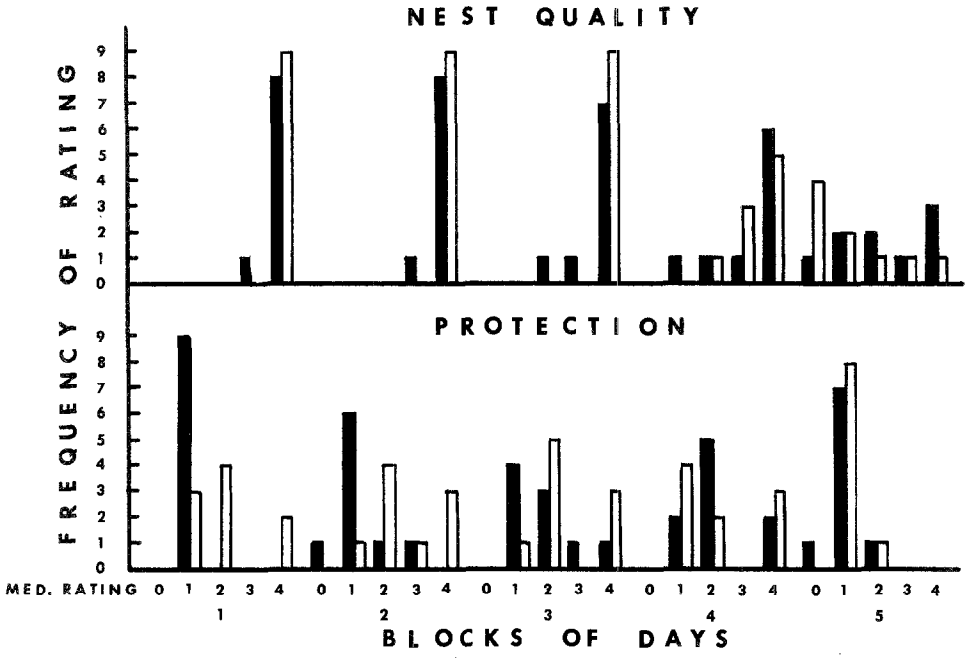

FIG. 3. Frequency of median ratings for nest quality and protection during the five 4-day blocks for the alcohol (shaded) and maltose (unshaded) dams.

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