Pharmacology Biochemistry & Behavior, Vol. 19, pp. 513–518, 1983. © Ankho International Inc. Printed in the U.S.A.

The Effects of Alcohol Induced Malnutrition in Pregnancy on Offspring Brain and Behavioral Development[1]

H. L. BARTLEY, I. R. COYLE AND G. SINGER[2]

Department of Psychology, La Trobe University, Bundoora, Australia, 3083

Received 24 February 1982

BARTLEY, H. L., I. R. COYLE AND G. SINGER. *The effects of alcohol induced malnutrition in pregnancy on offspring brain and behavioral development.* PHARMACOL BIOCHEM BEHAV 19(3) 513–518, 1983.—Alcohol is known to have various deleterious effects in all animals including man. The present study was designed to establish whether the effects of moderate EtOH intake during pregnancy on offspring are due to toxic effects of the substance or to nutritional changes; whether effects are long lasting or limited in duration; and whether effects are due to the prenatal action of the substance or effects persisting into the postnatal period. The findings show that the effects obtained in our study are due to malnutrition engendered in the prenatal period and are of limited duration. Since much evidence suggests that early deficits are difficult to compensate for, it is possible that the tests used with mature animals in this study may have been insensitive to residual deficits. Alternatively, rats may truly have compensated for early retardation. This does not necessarily imply that the same compensatory processes would apply in humans, where greater complexity of environmental demands is imposed from an early age.

Alcohol Prenatal effects

IN THE following studies, the effects of moderate intake of ethyl alcohol (EtOH) throughout the period of gestation were investigated. Recently the Acting Attorney General issued an advisory warning that pregnant women should not only abstain from alcoholic beverages but also should ". . . be aware of the alcoholic content of food and drugs." In a criticism of this statement and consequent action by the National Institute of Alcohol Abuse and Alcoholism [10] it was argued that evidence for the effects of moderate drinking on fetuses is not strong, in particular data from the very few human studies may be biased because of under-reporting of alcohol consumption by some of the women in these studies. In most animal studies, high alcohol levels are used [12] and it is argued by Abel [3] that the daily lowest threshold level for alcohol effects to appear is 150 mg/kg/day.

It has been pointed out [3] that many studies on prenatal effects of EtOH have been deficient on several methodological counts. First, despite known nutritional deficits following EtOH ingestion, in many studies no attempt has been made to assess nutritional factors in EtOH effects. Where pair-feeding has been included, it has not always been the case that ad lib controls are also included to allow assessment of interaction of drug and nutritional factors. Second, few studies include attempts to establish whether effects of EtOH carry over into the postnatal period. Third, methods of administration of EtOH have often been artificial and likely to be stressful (e.g., intubation, injection). Even though controls for these treatments may be included, it is again often the case that non-treated controls are absent, so precluding the possibility of assessment of drug × stress interactions.

The present studies were designed to overcome some of these defects. Both pair-fed and ad lib fed controls were used. Fostering and cross-fostering procedures were employed. Animals were given EtOH solution as their sole fluid source. Standard laboratory chow provided a balanced nutritional source.

In the first study, effects of prenatal EtOH on early development were examined. In the second study, the effects of prenatal EtOH on behaviour of mature (60 and 90 day old) offspring were studied.

EXPERIMENT 1: EFFECT IN RATS OF FORCED INTAKE OF EtOH DURING PREGNANCY ON DEVELOPMENT OF OFFSPRING

METHOD

Subjects

Seventy-one nulliparous female rats of the La Trobe strain of Wistar rats were used. Thirty-eight of these contributed litters which constituted subjects for developmental studies. Eleven litters were allocated to the alcohol-drinking (EtOH) group (4 unfostered (UF); 4 fostered (F); 3 cross-fostered (XF)). Ten litters were allocated to pairfed (PF) group (4 UF; 3 F; 3 XF). Seventeen litters were allocated to a water-drinking control (CON) group (4 UF; 5 F; 8 XF).

[1]This work was funded by the Australian Associated Brewers.
[2]Requests for reprints should be addressed to G. Singer.

TABLE 1
DESCRIPTION OF REFLEX TESTS

Reflex	Eliciting Stimuli	Response	Age in Days at Which Test Commenced
Righting	Rat placed on back on a flat surface	Turns over on to ventral surface	1
Negative geotaxis	Rat placed, head downwards, on a 20° slope	Turns to face up the slope	1
Cliff avoidance (cliff drop aversion)	Rat put on edge of bench, with nose and forefeet just over edge	Moves away from "cliff"	1
Free-fall righting (acceler-ation righting)	Rat dropped, back downwards, from 35 cm on to cotton wool pad	Turns in mid-air to land on all fours	10
Auditory startle	Sound stimulus; snap of mouse trap closing on wooden base	Sudden, brief extension of hind limbs (which raises hind-quarters)	10
Visual placing	Rat held upside-down near edge of bench	Lifts head and extends forelegs in direction bench	On day of eye open-ing

Adapted from Smart and Dobbing [22].

Procedures

Preparation of females. After at least seven day's habituation to laboratory conditions, females were matched for body weight, and allocated to one of the three experimental conditions. They were then placed with a male until mating, as determined by the presence of a vaginal plug, had occurred. If mating had not occurred within 10–12 days, females were removed from the sample.

Following mating, females were housed individually in plastic colony cages for the duration of pregnancy and lactation.

The "Etoh" group of animals was provided with a 6.8 v/v solution of 99% EtOH in tap water as their sole source of fluid. The free-fed control group (CON) were provided with tap water. Lab chow was provided ad lib to both of these groups. The remaining group (SUC) constituted a pair-fed control group. These rats were given sucrose solution isocaloric to EtOH in the same volume of fluid as that consumed by the matched EtOH subject, and daily food allowance was restricted to that amount consumed by the matched subject on the same day of pregnancy. In addition to measurement of food and fluid intake, body weights of all animals were measured daily throughout pregnancy.

As soon as possible after the birth of their offspring, experimental females were returned to ad lib food and water for the duration of the nursing period.

Twenty-four to thirty hours after birth (i.e., on Day 1), litters were culled to eight pups, with the maximum possible number of males in each litter. In addition, the brain of one of the culled males from each litter was removed (see below).

In the case of unfostered (UF) animals, litters were returned to the natural mother. In the fostered (F) condition, litters were exchanged between pairs of mothers of the same prenatal treatment condition, when those litters were born within 24 hours of each other and mothers were of similar (5 g) premating body weights. In the cross-fostered (XF) condition, litters were exchanged between pairs of mothers of similar premating body weight and gestation within 24 hr of each other. In this instance, however, offspring of EtOH pretreated mothers were placed with a water-pretreated mother, and vice-versa. Similarly, offspring of sucrose-pretreated and water-pretreated mothers were exchanged. All litters then remained with these mothers until weaning at 25 days of age.

Where litter size fell to five or less during the period of developmental testing, data for that litter were not included in the analysis. Litters of less than eight at Day 1 were discarded.

Neonatal brain weights. The weights of the cerebellum with associated brain stem, and cerebral hemispheres with associated tissue, for one male from each litter, were determined after fixing in glutaraldehyde.

Developmental measures. Commencing on neonatal Day 1, the following tests of reflex ontogeny were made daily, on male pups only (Table 1).

In all cases, presence or absence of response was scored. In the case of righting, negative geotaxis and cliff avoidance

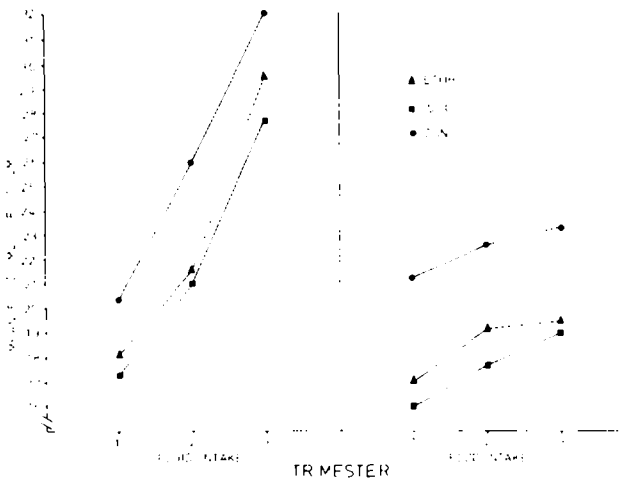

FIG. 1. Mean food and fluid intake of female rats in each trimester of pregnancy.

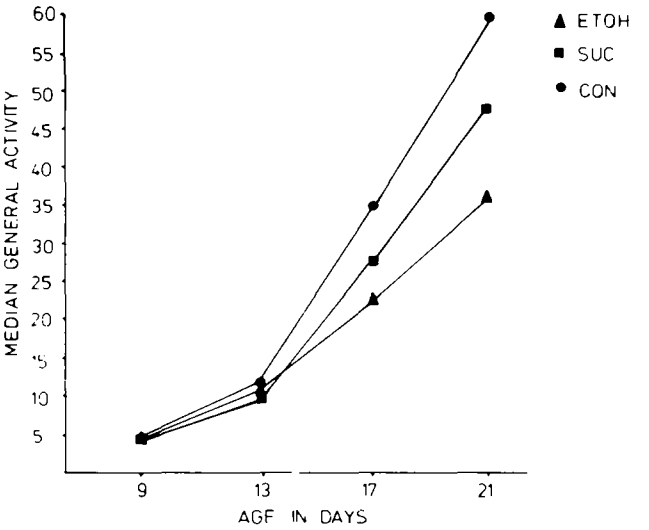

FIG. 2. Median general open field activity of rat pups over age.

a criterion of one minute to respond was set. For each behaviour, each animal was tested until the response was performed on three consecutive days, or until 21 days of age.

In addition, body weights were measured every 48 hr and pups were examined daily for maturation of three physical features viz appearance of upper incisors, eye opening and ear unfolding. When animals were 9, 13, 17 and 21 days old, exploratory behaviour was observed in a circular open field, 84 cm in diameter and 66 cm high. The floor in the centre of the apparatus was divided into 30 squares, each 10 cm square; the remainder of the floor was marked into 16 approximately equal areas. The interior surfaces of the apparatus were matt black.

Prior to reflex testing, each animal was placed in the centre of the open field and the following behaviours were recorded during a two minute observation period; number of areas entered; frequency of head lifting with both forelegs on the ground; frequency of head lifting with one foreleg on the ground (half-rearing); frequency of rearing on the hind legs either against a vertical surface or unsupported; frequency of turning (defined as a change in direction of 180 in a radius not exceeding one body length); and frequency of grooming. Incidence of urination and defecation were also noted. The apparatus was sponged clean and dried after each animal had been tested.

RESULTS

Prenatal Treatment Effects

Characteristics of pregnancy. Treatment groups did not differ in any aspect of pregnancy except percentage weight gain from initial to last day of pregnancy. CON animals gained significantly more weight than EtOH or SUC animals. (Mann-Whitney U test EtOH vs. SUC: U=56 NS; EtOH + SUC vs. CON: $z=5.0$ $p<0.001$).

Fluid intake of pregnant females differed significantly between groups, $F(2,35)=6.14$, $p<0.01$, and across trimesters of pregnancy, $F(2,70)=98.07$, $p<0.001$. Food intake also differed significantly between groups, $F(2,35)=15.38$, $p<0.01$, and across trimesters, $F(2,70)=24.66$, $p<0.001$. Mean intake of food and fluid in each trimester by each group is shown in Fig. 1.

In the sections below, statistical analyses consisted of Mann-Whitney U test comparisons between individual groups with $\alpha=0.01$, except where otherwise indicated.

Body weights of offspring. Body weight of developing rats showed a significant increase over age from 1–21 days, $F(19,204)=4377.39$, $p<0.001$. However, prenatal treatment groups did not differ, $F(2,204)=2.38$, $p>0.05$.

Neonatal brain weights. Mean whole-brain weights do not differ significantly, (EtOH vs. SUC: $t(17)=0.34$). Similarly, cerebellar percentage weights of EtOH and SUC pups do not differ. However, the pooled data of these two groups were significantly different from CON cerebellar percentage weights ($U=30$, $p<0.002$).

Physical indices of development. Ear unfolding occurred later in both EtOH and SUC pups than in CON pups (EtOH vs. CON: $z=4.12$, $p<0.001$; SUC vs. CON: $z=2.72$, $p<0.01$). Eruption of upper incisors occurred later in SUC than in CON pups, $z=5.05$, $p<0.001$. EtOH and CON groups did not differ significantly.

Commencement of eye opening occurred earlier in SUC pups than in EtOH pups, $z=3.98$, $p<0.001$. CON pups also preceded EtOH pups, $z=5.15$ $p<0.001$.

Behavioural indices. In no case were EtOH and SUC groups found to differ significantly from each other. When pooled data of these groups were compared with those of CON pups, however, significant differences were found on four of the six indices monitored. These were negative geotaxis ($z=2.97$, $p<0.005$), cliff avoidance ($z=3.16$, $p=0.001$), auditory startle ($z=2.83$, $p<0.005$) and visual placing ($z=4.55$, $p<0.001$). In all cases, the developmental sign appeared earlier in CON than in EtOH and SUC pups.

Open-field activity. It was found that at none of the ages tested—9, 13, 17 and 21 days of age—did EtOH and SUC groups differ significantly in activity level (sum of component activities). At 9 and 13 days the pooled data of these groups did not differ from the CON group. However, at 17 days ($z=3.13$, $p<0.001$) and at 21 days ($z=3.43$, $p<0.001$) the CON group was significantly more active. The median activity score at each age for each treatment group is shown in Fig. 2.

Frequency of urination decreased with age in all groups. Treatment groups differed only at 17 days of age when a

greater number of SUC pups urinated than of EtOH pups ($z=6.79$, $p<0.01$). SUC and CON groups were not significantly different.

Frequency of defecation was low at all ages, and treatment groups did not differ significantly.

Fostering Effects

Only minor differences in physical and behavioural developmental indices were found between fostered, cross-fostered and unfostered groups. These differences were within treatment groups and do not affect the between-group findings.

DISCUSSION

From the data reported above, it appears that consumption of moderate amounts of alcohol throughout pregnancy has effects on aspects of physical and behavioural development. The data show that these effects are due to variation of the nutritional status of the mother during pregnancy, since performance of the EtOH and SUC groups was similar throughout, and patterns of ingestion of EtOH and SUC mothers during pregnancy were similar, but differed from those of the CON mothers. These data differ from those obtained in a series of studies [15, 16, 17] in which performance of offspring of rats pair-fed with sucrose was found to differ from that of EtOH-fed animals, and to be similar to ad lib fed animals. However, animals in their pair-fed and EtOH conditions were fed liquid diets throughout. This procedural difference may account for the difference in findings.

The effects observed appear to be due to prenatal influences only, since no clear effects of cross-fostering, which would indicate residual postnatal effects, were obtained. This is in accordance with data reported by Osborne *et al.* [12] who used very much higher doses of alcohol. Relatively few studies have been aimed at resolution of this question. In one study in which behavioural effects of prenatal EtOH were found [1] offspring of both EtOH and water pretreated rats were placed with water pretreated mothers. Thus the effects observed are attributable to the prenatal effects of EtOH. However, this design leaves open the question of whether postnatal effects are also present. The absence in the present study of differences due to cross-fostering suggests that neither carry-over EtOH effects on offspring nor changes in maternal behaviour consequent on prenatal EtOH treatment occur in the postnatal period. Absence of prenatal EtOH effects on maternal behaviour has also been reported in mice [9].

The second experiment, reported below, was directed toward the question of whether early effects persist to maturity. Spontaneous alternation performance and learning of complex maze tasks were investigated.

EXPERIMENT 2: EFFECT IN RATS OF FORCED INTAKE OF ETHANOL DURING PREGNANCY ON SPONTANEOUS ALTERNATION AND HEBB-WILLIAMS MAZE PERFORMANCE OF MATURE OFFSPRING

A question was not resolved by the data obtained in the first experiment which relates to the persistence of early effects of prenatal EtOH.

The second experiment was directed toward this question. Spontaneous alternation performance (cf., [16]) and learning of Hebb-Williams maze tasks were investigated. The latter was selected as it is sensitive to early environmental variation [11, 12, 20] and provides a measure of general cognitive functioning [14].

An earlier study [6, 7, 18] showed that imipramine taken by rat mothers during pregnancy will affect the behaviour and brain morphology of the offspring. However, these effects are only manifest if the offspring are reared in an enriched environment. That is, offspring of placebo treated mothers show improved performance and brain development in the enriched environment whereas offspring of the imipramine treated mothers do not benefit by exposure to this environment. In the normal colony environment, there is no difference in either the behaviour or the brain development of the imipramine treated and control offspring.

In the present experiment, therefore, subjects from each of the three prenatal treatment groups (alcohol, sucrose or water) were allocated either to an enriched or to a deprived environment for 30 days after weaning. Animals were tested at maturity (60 and 90 days of age).

METHOD

Subjects

Fifty nulliparous females of the La Trobe strain of Wistar rats were used. Twenty-seven of these contributed litters which were the subjects for this study. Ten litters were allocated to the EtOH group (5 each to the enriched (EC) and deprived (DC) environments). Six litters were allocated to the pairfed condition (3 each to EC and DC), and 11 litters to the water-drinking control condition (5 to EC; 6 to DC).

Procedures

Preparation of females. This was essentially as described for the previous study.

At weaning, female offspring were killed and males housed with litter mates in colony cages in groups of 2–4. At 30 days of age, half of the litters in each treatment group were placed in Environmental Enrichment cages (EC). These cages measured 130×100×100 cm. Walls and the hinged roof were constructed of clear acrylic, while the floor was of wire mesh. The cage contained a variety of objects including a milk crate, a running wheel and a climbing arc. No more than 16 animals were housed in one cage. Litters housed together did not differ in age by more than 3 days. Litters maintained on relatively deprived conditions (DC) were left as they had been organised at weaning.

All litters were left undisturbed except for routine maintenance until 60 days of age, at which time EC animals were returned to colony-box housing and all animals were tested for spontaneous alternation.

Behavioural Testing

Spontaneous alternation. Spontaneous alternation was tested using a T-maze. The walls and floor of this were constructed of clear acrylic. The roof was hinged to permit easy placement and removal of the animals. Each area of the maze measured 36 cm long. The approach alley was 100 cm long. All areas were 12 cm wide and 12 cm high. Guillotine-type acrylic doors were placed at the commencement of choice arms of the maze to prevent retracing. Another door was located 25 cm along the start alley.

Each animal was placed at the commencement of the starting alley. When it had made a four-footed entry into one of the arms of the maze it was immediately removed, replaced in the starting alley and given a second trial using the same procedure and criterion. The time taken to enter one of

the arms, together with the arm entered, was recorded for each trial. If the animal entered the same arm on both trials it was classified as having perseverated. If it entered a different arm on each trial it was classified as having alternated. If the animal did not enter either of the arms within five minutes, it was classified as having failed to respond. After each animal had been tested, the paper covering the floor of the maze was replaced so as to minimise variability in olfactory cues.

Hebb-Williams maze learning. The method in this instance incorporates modifications by Thompson and Kano [20] and Petit and Isaacson [13] to the method of Rabinovitch and Rosvold [14]. The apparatus consisted of a box with an entrance alley and food compartments at opposite corners of an open field. This box was housed on a table. All parts of the apparatus were painted flat grey. The floor was marked into 36 squares in area 12 cm. These were outlined by grooves in the floor into which partitions marking maze boundaries were fitted, and which served to define error zones. The procedure involved four phases. In the first of these, preliminary preparation of the animals occurred. In the second phase, animals were habituated to the appartus; in the third phase, they were given a series of practice problems; and in the fourth phase, test problems were given. The four phases are described in greater detail below.

Phase 1: Preliminary Preparation

Commencing at age 83 days, normal laboratory chow was removed and wet mash substituted. After two days on this diet, animals were 23 hour food deprived. Wet mash was available for 1 hr per day as the sole food source; tap water was however available ad lib as fluid source.

Phase 2: Habituation

After two days on the 23 hour deprivation schedule, animals were placed in the apparatus in litter mate groups (normally 5–6 animals) at the time at which feeding normally commenced. No barriers were present in the apparatus. Animals were permitted freely to explore the apparatus, and to eat wet mash available in the food box, for a half-hour period, after which animals were permitted to eat wet mash for a further half-hour in a holding box before being returned to home cages. This procedure was followed for a total of three days. Following Rabinovitch and Rosvold [14], animals were handled frequently during this time.

Phase 3: Practice Trials

On the seventh day (age 90 days) animals were weighed and individual training began on the practice problems. A different practice problem was used each day. The time required for the animal to reach the goal box was recorded for each of the six trials on a given problem. A maximum of three minutes per trial was allowed. When an animal had entered the goal box it was allowed to eat for an average of 20 seconds before commencement of the next trial. Following the six trials on a problem, animals were allowed to eat for 30 minutes in a holding box before being returned to the home cage. The apparatus was wiped clean with a cloth soaked in a strong detergent solution after each animal had completed its series of six trials.

This procedure was followed daily until an animal reached the criterion of completing all of the six trials on a problem in a total of 30 seconds or less. If all six of the practice problems were completed before the criterion was reached, practice problem 1 and following were represented on subsequent days, again one problem per day with six trials per problem. After 12 days and 24 days on practice problems without reaching criterion, animals were given another half-hour habituation trial (Phase 2) before practice problem 1 and following were presented again. If after 30 days of practice problems criterion was not reached the animal was discarded from the sample.

Phase 4: Test Trials

Twenty-four hours after criterion was reached, animals were given the first of six test problems. The problems used were numbers 3, 5, 6, 9, 10, 12 [12]. Six trials were given on each problem, and only one problem was presented per day. As in Phase 3, animals were permitted to eat for an average of 20 seconds in the goal box after each trial, and on completion of the six trials were fed outside the home cage for a further 30 minutes. The time taken, and the number of errors made (cf., [14] p. 125) was recorded for each problem. On completion of the last problem animals were returned to ad lib food.

Brain weights. For one or two males from each litter, whole brain weights, the weights of cerebellum with associated brain stem, and cerebral hemispheres with associated tissues, were determined.

RESULTS

Characteristics of Pregnancy

Characteristics of pregnancy were the same as reported in Experiment 1.

Spontaneous Alternation

Immediately prior to testing of spontaneous alternation at 60 days of age, body weights of animals were taken. No significant differences were found between EC and DC subgroups of the three prenatal treatment groups. However when EC and DC groups were pooled it was found that CON animals weighed significantly more than EtOH and SUC groups. Planned contrast EtOH vs. SUC: $F(1,139)=0.57$, NS; planned contrast EtOH=SUC vs. CON=4.58, $p<0.05$.

The frequency of alternation, perseveration and nonresponse on the test of spontaneous alternation were compared. When data of EC and DC animals were analysed with prenatal treatment groups pooled, a significant difference was found, [2] $z=6.42$, $p<0.05$. EC animals made more alternation responses than DC animals. No differences were found between prenatal treatment groups.

Hebb-Williams Maze Learning

Body weights at 90 days of age. No differences were found between EC and DC groups in body weight. Prenatal treatment groups also did not differ significantly, although the difference between EtOH + SUC and CON groups approached the 0.05 level (CV for $F=3.92$; $F=3.5$).

Number of practice trials taken to reach criterion. No differences were found in number of practice trials taken to reach criterion between EC and DC animals in the case of EtOH and SUC groups. However in the case of CON animals, the EC group took significantly fewer trials to reach criterion than did DC animals (Mann-Whitney U test, $z=6.37$, $p<0.001$). When CON:EC animals were compared with EtOH and SUC groups no significant differences were found. Thus CON:DC animals were slower than all other groups to reach criterion on practice trials.

Number of errors made on test trials. The mean number of errors per problem made by each animal was used as the basis for analysis.

No significant differences were found between any of the groups.

Brain weights at 90 days of age. The whole-brain and cerebellar weights of animals in each treatment group did not differ significantly, although there was a non-significant tendency for brains of EC-reared SUC and CON animals to weigh more than their DC controls.

DISCUSSION

The data from our experiments show clearly that moderate intake of EtOH during pregnancy leads to a number of developmental and behavioural deficits in young offspring. Comparison with a pair-fed sucrose and a normal water drinking control group show that these effects are the result of malnutrition. This may be due to the dose of EtOH used; with higher doses of EtOH toxic effects may occur with or without malnutrition. The findings of the present study show that consumption of moderate amounts of alcohol throughout pregnancy has effects on physical and behavioural development similar to malnutrition produced by an equicaloric sucrose diet where malnutrition was induced through pairfeeding. In most respects the present study is similar to a study reported by Osborne *et al.* [12] except that the latter experimenters used a much higher daily dose of alcohol for part of the gestation period and although the differences between pair-fed and alcohol rats are different for the two studies other findings are consistent. Thus, comparisons of unfostered, fostered and cross-fostered offspring in both studies show that the observed effects were prenatal and that no effects which could be attributed to residual toxicity or changed maternal behaviour were observed.

Relatively few studies have investigated the long-term effects of prenatal EtOH. Animals intermediate in age between the two groups (developing and mature) in the present study show prenatal EtOH effects on passive avoidance [15], spontaneous alternation, active avoidance [16] and exploratory behaviour [17], when higher doses of EtOH than at present were used.

Data from the second experiment showed that there were no behavioural effects after 90 days and also that the offspring of all mothers regardless of treatment were able to benefit by the enriched environment. This general enrichment effect was unlike the findings from earlier studies [6,7] that the offspring of imipramine treated mothers could not benefit from an enriched environment when compared to the offspring of placebo treated mothers. The limited duration of EtOH effects may have been the result of dose level. Bond [4] has shown that consistent EtOH effects which persist into adulthood are obtained with much higher doses than those used in the present study. Alternatively compensation for earlier deficits may occur for these effects of malnutrition and may not readily occur when toxic effects are present. It is also possible that residual behavioural deficits are the result of the absence of cumulative learning and that one test may be insufficient to detect subtle deficits [8].

While some evidence shows that the Hebb-Williams maze is sensitive to early-life manipulations [11, 13, 20] it did not reflect prenatal EtOH effects in a study in which larger doses were used, and further where other behavioural indices were affected [4]. Differential sensitivity of tasks to EtOH effects has also been shown by Abel [2]. In general the tests selected here may not be designed for specific functions and may be irrelevant for others which could have been effected by the drug.

ACKNOWLEDGEMENTS

The assistance of E. Marshall is gratefully acknowledged.

REFERENCES

1. Abel, E. L. Effects of ethanol on pregnant rats and their offspring. *Psychopharmacology (Berlin)* **57:** 5–11, 1978.
2. Abel, E. L. Prenatal effects of alcohol on adult learning in rats. *Pharmacol Biochem Behav* **10:** 239–243, 1979.
3. Abel, E. L. Procedural considerations in evaluating prenatal effects of alcohol in animals. *Neurobehav Toxicol* **2:** 167–174, 1980.
4. Bond, N. Prenatal alcohol exposure in rats: A review of its effects on offspring activity and learning ability. *Aust J Psychol* **33:** 331–344, 1981.
5. Bond, N. and E. Di Giusto. Avoidance conditioning and Hebb-Williams maze performance in rats treated prenatally with alcohol. *Psychopharmacology (Berlin)* **58:** 69–71, 1978.
6. Coyle, I. R. and G. Singer. The interaction of postweaning housing conditions and prenatal drug effects on behavior. *Psychopharmacologia* **41:** 237–244, 1975.
7. Coyle, I. and G. Singer. The interactive effects of prenatal imipramine exposure and postnatal rearing conditions on behavior and histology. *Psychopharmacologia* **44:** 253–256, 1975.
8. Coyle, I. R., M. J. Wayner and G. Singer. Behavioral teratogenesis: A critical evaluation. In: *Advances in the Study of Birth Defects vol 4: Neural and Behavioral Teratology*, edited by T. V. N. Persaud. Baltimore: University Park Press, 1980.
9. Ewart, F. and M. Cutler. Effects of ethyl alcohol on behaviour in nursing female mice. *Psychopharmacology (Berlin)* **66:** 143–146, 1979.
10. Kolata, G. B. Fetal alcohol advisory debated. *Science* **214:** 642–645, 1981.
11. McCullough, M. and D. Blackman. The behavioural effects of prenatal hypoxia in the rat. *Dev Psychobiol* **9:** 335–342, 1976.
12. Osborne, G. L., W. F. Caul and K. Fernandez. Behavioral effects of prenatal ethanol exposure and differential early experience in rats. *Pharmacol Biochem Behav* **12:** 393–401, 1980.
13. Petit, T. and R. Isaacson. Anatomical and behavioral effects of colchicine administration to rats late in utero. *Dev Psychobiol* **9:** 119–129, 1976.
14. Rabinovitch, M. and H. Rosvold. A closed field intelligence test for rats. *Can J Psychol* **5:** 122–128, 1951.
15. Riley, E., E. Lochry and N. Shapiro. Lack of response inhibition in rats prenatally exposed to alcohol. *Psychopharmacology (Berlin)* **62:** 47–52, 1979.
16. Riley, E., E. Lochry, N. Shapiro and J. Baldwin. Response perseveration in rats exposed to alcohol prenatally. *Pharmacol Biochem Behav* **10:** 255–259, 1979.
17. Riley, E., N. Shapiro and E. Lochry. Nose-poking and head-dipping behaviors in rats prenatally exposed to alcohol. *Pharmacol Biochem Behav* **11:** 513–519, 1979.
18. Singer, G. and I. R. Coyle. The effect of imipramine administration before and during pregnancy on litter size in the rat. *Psychopharmacologia* **32:** 337–342, 1973.
19. Smart, J. L. and J. Dobbing. Vulnerability of developing brain. II. Effects of early nutritional development on reflex ontogeny and development of behaviour in the rat. *Brain Res* **28:** 85–95, 1971.
20. Thompson, W. and K. Kano. Effects on rat offspring of maternal phenylalanine diet during pregnancy. *Psychiatry Res* **3:** 91–98, 1965.