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Viability and Sensorimotor Development of Mice Exposed to Prenatal Short-Term Ethanol[1]

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FISH, B. S., S. A. RANK, J. R. WILSON AND A. C. COLLINS. *Viability and sensorimotor development of mice exposed to prenatal short-term ethanol.* PHARMAC. BIOCHEM. BEHAV. 14(1) 57–65, 1981.—The identification of the fetal alcohol syndrome in man has led to an abundant, but sometimes contradictory body of research examining the effects of prenatal ethanol exposure in animals. The present study examines the early sensorimotor development of heterogenous stock mice after ethanol exposure during one of two time periods in gestation, days 8–12, or days 14–18. Animals received either a liquid diet containing 20% ethanol derived calories, an isocalorically balanced yoked control diet or a lab chow control diet. Results indicated no apparent effects due to administration of alcohol during the middle of gestation, but a marked decrease in offspring viability, birthweight, and growth due to administration during the last part of gestation. The late gestation treatment group also showed a slight delay in the rate of development of the grasping and horizontal screen tasks. Overall, animals that survived showed relatively normal sensorimotor development.

Ethanol Prenatal Fetal alcohol syndrome Fetal alcohol effect Sensorimotor development Viability Critical periods Mice

NUMEROUS clinical reports have documented and described a pattern of multiple congenital anomalies in the offspring of women who consumed alcohol during pregnancy. This pattern of abnormalities, labeled the Fetal Alcohol Syndrome (FAS), is characterized by pre- and post-natal growth deficits, craniofacial, limb and joint, external genitalia and cardiac abnormalities and impaired motor and mental functioning. Autopsy findings have been reported for two FAS infants who died between 0 and 6 weeks of age [12] and for one who died at 9 months of age [29]. Sheets of aberrant neural and glial tissue covering part of the brain surface, disorganized neuronal arrangements within the brain tissue, small and disorganized cerebellum, ventricular dilation and agenesis of the corpus callosum and anterior commissure were among the neurological findings.

However, many clinical researchers have failed to observe the complete FAS in the offspring of women ingesting significant amounts of alcohol during pregnancy. Rather, they describe components of the syndrome or Fetal Alcohol Effects (FAE) (e.g. [38]). The most frequent problem noted in these children is deficient intellectual performance [19] measured longitudinally by standard intelligence tests [50]. It has been reported that some children of alcoholic mothers are mildly retarded in the absence of other FAS features [48]. Consequently, it has been hypothesized that mental retardation may be the most sensitive manifestation of alcohol ingestion during pregnancy [34].

There have been several indications that the quantity of alcohol consumed may not be directly related to the FAE [21,29]. An inverse relationship between the level of alcohol intake and the severity of abnormalities in the offspring has been reported along with calculations that the degree of the effect was unrelated to the length of alcohol abuse by the mother [29]. Some children with FAE were born to women who occasionally consumed large quantities of alcohol, but were not classified as chronic alcoholics [7,12]. Infants born to "social drinkers" have been found to be deficient in muscle tonicity, habitation, and alertness [49] and to display, in reduced form, other behaviors characteristic of FAS children [25].

These studies are replete with the problems associated with the use of human populations such as lack of control over critical variables (nutrition, multidrug use, socioeconomic status, medical care), difficulty in assessment of amount and time of ethanol consumption and in determination of treatment effects (e.g. IQ). However, the clinical reports suggest a number of hypotheses concerning the effects of prenatal exposure to ethanol which are amenable to laboratory investigation. For example, since the amount of alcohol consumed may not predict FAE, maternal absorption, metabolism or excretion of alcohol may be crucial. The amount of ethanol (or its metabolites) in the maternal system at any given time may be important rather than the total amount consumed. Since duration of alcohol consumption

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may not predict FAE, one or more different critical periods for ethanol consumption during pregnancy are indicated. The fact that *in utero* ethanol exposure can produce mental retardation in the absence of other FAS features indicates that alcohol may be a behavioral teratogen—i.e., at some doses, it may result in behavioral dysfunctions in the absence of observed physical abnormalities [18]. In addition, the intellectual deficits imply that ethanol can selectively affect the central nervous system (CNS). In fact, one of the early autopsy reports described CNS abnormalities in the absence of any external clinical manifestations of exposure to ethanol prenatally [12].

Many animal studies assessing the effects of relatively large levels of ethanol administered during gestation have been successful in eliciting some of the morphological alterations described in the human literature [11, 22, 41, 54] including CNS abnormalities [10, 11, 14, 24, 42, 43, 53]. Behavioral studies associated with large levels of ethanol are sometimes contradictory [8, 10, 46] although many indicate task-dependent impairments in learning abilities [2, 9, 31, 44, 45]. Studies in which relatively low levels of ethanol are administered do not usually produce the physical dysmorphology associated with the FAS; behavioral findings are frequently inconsistent [1, 13, 23, 44, 45, 57, 58, 59]. For example, locomotor activity of treated offspring has been reported to be increased [23], decreased [57] or unchanged (Fish, in preparation) following low level ethanol administration to pregnant mice. Some of the behavioral inconsistencies may be due to strain or species used, variations in duration, concentration and method of ethanol administration, age at the time of testing and tasks utilized for testing behaviors. Many of the studies have not employed adequate control groups or cross-fostering procedures.

Since more women consume low rather than high levels of ethanol during pregnancy [51] studies utilizing relatively low levels of ethanol are essential. Moreover, rarely has the critical period issue been specifically addressed in laboratory investigations. It would appear that the latter half of mouse gestation would be of particular interest since this is the period during which the major portion of prenatal brain development occurs [5,26]. Given the mental retardation associated with fetal alcohol exposure, the learning and memory capacity of offspring prenatally exposed to ethanol should be of interest. Initially, however, sensory and motor capabilities should be evaluated since inferences about learning and memory may be spurious if they are confounded by dysfunctional sensory or motor abilities which are necessary for task performance.

The present study is an attempt to determine the effects on early mouse development of relatively low levels of ethanol during critical gestation periods using pair fed as well as lab chow fed controls, along with cross-fostering methods. The level of ethanol administered was selected on the basis of doses found to be nonteratogenic in previous reports [40,59] and pilot studies utilizing both inbred strains and heterogenous mouse stocks. The pattern of consumption associated with a liquid diet was considered to be conducive to maintaining low maternal ethanol levels [30]. A control group which is fed the same proportion of diet consumed by the ethanol treatment animals with the ethanol component replaced by an isocaloric agent (e.g. sucrose) is essential to the analysis of the effects of ethanol. Differences between this pair fed or yoked control group and the ethanol group may be attributed to the ethanol rather than to dietary factors. However, the yoked control group cannot be considered as an "untreated group" since a decrease in dietary intake induced by ethanol may result in an insufficient diet being administered to the yoked group. Lack of group differences might obscure the effects of malnutrition. Group differences might be due to a combination of ethanol treatment and malnutrition in the ethanol treatment group. The addition of a third group, an untreated (ad lib lab chow fed) group permits a determination of the dietary contribution to the measures that are assessed. Cross-fostering of the neonates to nontreated surrogate mothers standardizes the postnatal environment and allows for an assessment of treatment effects on the offspring independent of any effects that the treatments may have had on maternal behavior. Differences in maternal behavior could influence offspring development.

METHOD

Seventy-eight male and female genetically heterogenous (HS) stock [33] mice, approximately 80–90 days old, were set up in thirty-nine mating pairs with one female and one male per cage. The mice were maintained on a twelve hour light/dark cycle (lights on 7 a.m. to 7 p.m.) with free access to food (Wayne Sterilizable Lab Blox) and water. All of the females had given birth to one litter previous to this study.

The weight of all females was recorded at the beginning of the study. Females were checked twice daily (a.m. and p.m.) for the presence of a vaginal plug indicating the occurrence of copulation. The day of vaginal plug detection was designated as day one of gestation. Males were removed from the cages on day five of gestation. Weights for the females were recorded on alternate days beginning on gestational day ten.

The pregnant mice were assigned to one of three treatment conditions—(1) alcohol diet—Group A; (2) yoked diet—Group YC; or (3) lab chow control—Group C. Based on the same date of plug detection, twenty pregnant mice were designated as part of an alcohol-yoked control pair, while the remainder made up the lab chow control group (19 pregnant mice). Within the alcohol-yoked control pairs, mice were randomly assigned to one of two time periods for the administration of the diet, either in the middle of gestation (days 8–12—Group 1) or at the end of gestation (days 14–18—Group 2). This arrangement of treatments yielded a 2×3 factorial design with two independent variables—treatment time (Group 1 or Group 2), and type of treatment (Group A, Group YC, or Group C).

Animals in Group A received as their sole dietary intake during the treatment period, a liquid diet containing 20% of the calories provided by ethanol (approximately 3.5%). The diet, obtained from Bio-Serve, Frenchtown, NJ, contains all necessary nutrients for the pregnant mouse. The yoked control diet is isocalorically balanced with the ethanol diet and contains the same nutrients, with sucrose replacing the caloric equivalent of the ethanol. Pilot studies determined that the HS mice would consume the diet voluntarily, thus eliminating the need for injection or intubation of the ethanol. The diets were prepared from dry ingredients approximately every three days and were refrigerated until use. Diets were presented to the mice in inverted 25 ml graduated feeding cylinders.

Since it was critical to match delivery dates of alcohol-yoked control pairs in order to foster same age pups to a surrogate mother, a modification of the usual pair-feeding procedure was used. Matched sets of animals were started on the diet at the same time. For Groups 1A and 1YC, 20 ml

of the designated diet were first made available in place of lab chow and water on the evening of the eighth day. Feeding tubes were inspected morning and evening, and a fresh supply of diet was provided at both inspections. Measurement of consumption was recorded to the nearest 0.2 ml. A record of the cumulative amount consumed by each Group A animal dictated the amount of diet to be made available to their Group YC pair. Equivalent amounts of diet were to be consumed over the treatment period. The diet administration continued for five days ending on the morning of the 13th day. This procedure was repeated for Groups 2A and 2YC animals with treatments beginning on the evening of the 14th day and ending on the morning of the 19th day. After finishing the five day treatment period, animals were allowed free access to the lab chow and water until giving birth on day 21. Treatment was ended at the times indicated to insure that the ethanol would be eliminated from the mother's system at birth. Thus, if the neonates nursed immediately after birth, they would not be ingesting ethanol postnatally via the mother's milk.

At birth, a partial cross-fostering procedure was employed. In order to differentiate treatment groups (A, YC, C) all pups belonging to a condition were marked internally by the injection of one of three nontoxic dyes into the hindpaw. Experimental litters were culled to six of the animals that appeared healthiest (based on color, weight and movement), to encourage the highest possible survival rates, a requirement for the daily long-term testing of neonates. All pups were then given to lab chow-fed mothers who had undergone no experimental manipulations. Out of a group of four mothers—two lab chow and one alcohol-yoked control pair—two "families" of nine pups each were created. All pups of a given lab chow mother were removed and the dam received three Group A pups, three YC pups and three Group C pups from another lab chow mother. This second lab chow mother received the remaining three pups of the Group A litter, three pups of the Group YC litter and three of the pups born to the other lab chow dam.

Because of the timing of the births and the need to cross-foster within a 24 hr period to maintain consistency, nine litters (families) were derived from Group 1 and eight litters were formed from Group 2 treatment groups. All developmental tests were performed on these seventeen litters.

Testing Period

Offspring were tested every morning on days 2–20 on a number of developmental tests, adapted from Fox [16] and Wahlsten [55]. A brief description of the tests and the scoring methods used follows. In all cases, the adult response received the highest score, and the least developed response received the lowest score. If all three animals in a given subgroup (A, YC or C) of the litter demonstrated the adult response for three consecutive days, they were considered to have reached the criterion level of performance and testing on that particular task was terminated.

Weight

The birthweight of each animal in the entire litter was recorded before the animals were marked and cross-fostered. Beginning on day 2, animals were weighed daily to the nearest tenth of a gram until day 20.

Movement

The animal was placed on a flat surface and activity was observed for a 1 minute period. Four movement types were identified. 0—No movement; 2—Pivoting to the right or left; 4—Pivoting combined with forward movement in a straight line; 6—Immediate forward movement in a straight line.

Righting Reflex

The animal was placed on its back and the number of seconds required until righting was recorded. Ten seconds was the maximum time allowed for this task.

Cliff Aversion

The animal was placed on the edge of the counter with his body weight supported, but both front paws over the edge. The following responses were recorded: 0—No movement; 1—Forward movement; 2—Backward movement away from the edge.

Grasping Reflex

The animal was held by the experimenter and the forelimb and hindlimb were stroked with the shaft of a cotton swab (2.5 mm). The grasping response was noted. 0—No grasp; 1—Grasping with front paws only; 2—Grasping with both front and hindpaws.

Vertical Screen Test

The animal was placed on a vertical screen and the number of seconds that the animal could hold on, supporting its own weight, was recorded. Thirty seconds was the adult criterion level.

Horizontal Screen Test

The animal was drawn across a horizontal screen by the tail. 0—Animal offered no resistance; 1—Animal grasped screen, offering substantial resistance.

Auditory Startle

The experimenter clapped his hands several inches away from the animal and recorded the day that immediate "freezing" or flight was observed.

Ears Open

The day that the auditory canals were fully open was recorded.

Eyes Open

Day of eye opening was recorded on this scale: 0—Both eyes closed; 1—One or both eyes partially open; 2—Both eyes fully open.

Statistical Procedures

Because individual marking (in addition to the group markings) of the animals was not practical, the scores of the three animals making up a subgroup in each litter were combined to give one score per group per litter per day. In addition, use of the subgroup score limits genetic confounding that may occur with individual animal scores. Since some subgroups were missing one or two animals due to neonatal

deaths (see Results section), mean scores were determined for each subgroup. Within a litter, all members of a subgroup were required to perform a task at the adult level before the criterion level was reached for that group. The criterion level was reached by all groups for all tests by day 20 with one exception. These animals, for statistical purposes only, were assumed to reach criterion by day 21. Analyses of variance were used except where noted. The null hypothesis was rejected at $p>0.05$ for all statistical tests.

RESULTS

Because the amount of yoked control diet provided was dictated by the ethanol diet consumption, the mean intake levels for groups were fairly equal (Groups 1A and 2A $\bar{x}=88.45$ cumulative ml; Group 1YC and 2YC $\bar{x}=84.45$ ml). Groups 2A and 2YC consumed significantly more, $F(1,17)=17.008$, $p<0.01$, diet than did Groups 1A and 1YC ($\bar{x}=95.31$ ml vs. $\bar{x}=77.6$ ml). Group 1A consumed an average of 18.75 g/kg/day in comparison to the 24.94 g/kg/day intake of Group 2A, $F(1,8)=10.109$, $p<0.025$. As Fig. 1 illustrates, the diurnal pattern of diet consumption differentiated the two groups.

Group 2A consumed significantly more during the daytime (AM) than did Group 1A, $F(1,8)=37.75$, $p<0.001$; 3.63 g/kg/AM vs 8.44 g/kg/AM for Group 1A vs Group 2A. Analysis of the evening (PM) diet consumption indicated differences in the two treatment groups which appeared to be related to the duration of diet administration, Days×Group Interaction: $F(4,32)=6.68$, $p<0.001$. Subsequent analysis indicated that Group 2A consumed more than Group 1A on the first three evenings of diet administration and less than Group 1A on the last evening. The two groups did not differ in the amount of diet consumed on the fourth evening.

Weight gain for the pregnant females was fairly consistent across all groups. The mean weight gain for each group is presented in Table 1. Weight gain was computed by subtracting the original weight of the mouse from her weight on the day before giving birth. No significant differences between groups were found.

No maternal deaths occurred during the course of the study, but neonatal deaths (death other than stillborns) were observed (see Table 2). A chi square analysis performed on the frequency of neonatal death was significant, $\chi^2(2)=36.32$, $p<0.01$. A post hoc comparison revealed the frequency of Group 2A related deaths to be significantly different from the Group 2C and Group 2YC frequencies, $\chi^2(1)=7.5825$, $p<0.01$. There were no differences among Group 1 treatment groups in the number of neonatal deaths. The deaths found in Group 2A occurred in a seemingly random pattern of days throughout the preweaning period with approximately 50% of the animals dying during the first half of the period and 50% during the second half.

Litter size did not appear to be affected by the experimental manipulations as there were no significant differences between groups. A two factor (type of treatment, time of treatment) analysis of variance performed on birthweight did indicate a significant treatment type × treatment time interaction, $F(2,29)=3.33$, $p<0.05$. Subsequent analyses indicated that animals of Group 1 did not differ from one another. Group 2A birthweights were found to be significantly different from those of Group 2C birthweights. Group 2YC birthweights were not significantly different from Group 2C or from Group 2A. Group 2A birthweights were significantly

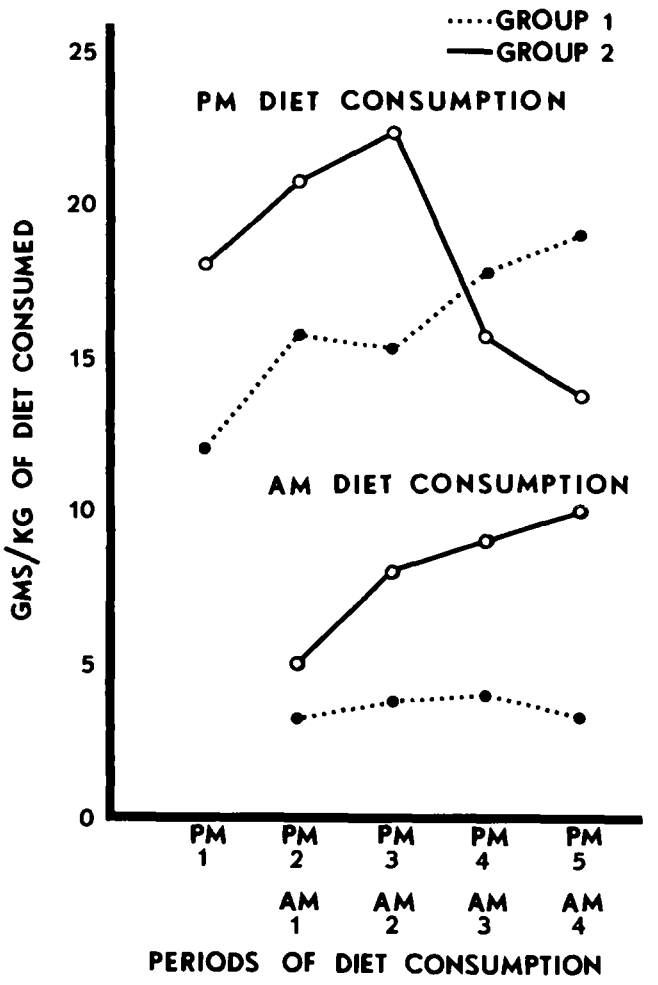

FIG. 1. Mean liquid diet consumption of ethanol-treated pregnant rats as a function of gestational treatment time and daily periods.

TABLE 1
MEAN MATERNAL WEIGHT GAIN IN GRAMS

	Group A	Group YC	Group C
Group 1	23.6	23.4	19.8
Group 2	21.9	19.0	22.6

TABLE 2
FREQUENCY OF NEONATAL DEATH

	Group A	Group YC	Group C
Group 1 Lived	25	26	26
Group 1 Died	2	1	1
Group 2 Lived	13	18	22
Group 2 Died	12	5	2

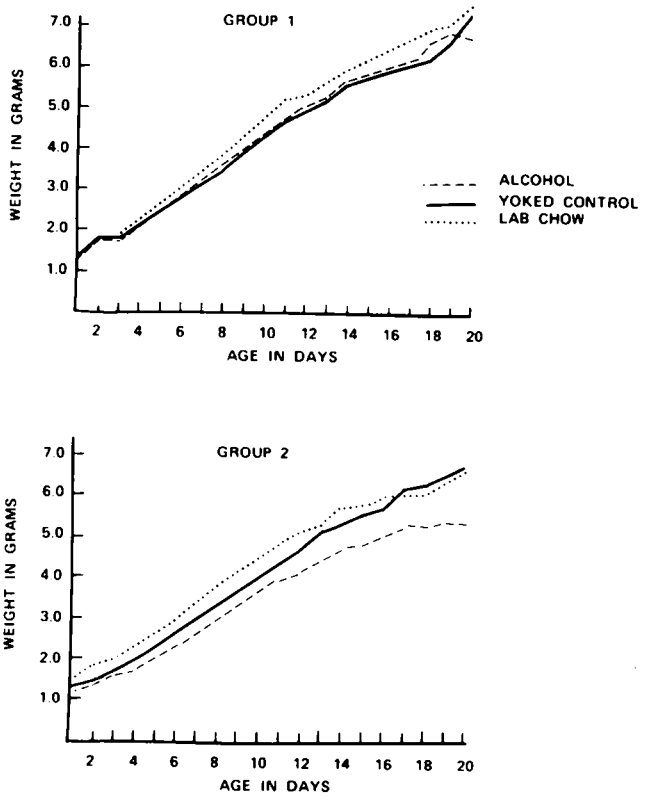

FIG. 2. Mean weights of prenatal ethanol treatment and control mice as a function of postnatal age.

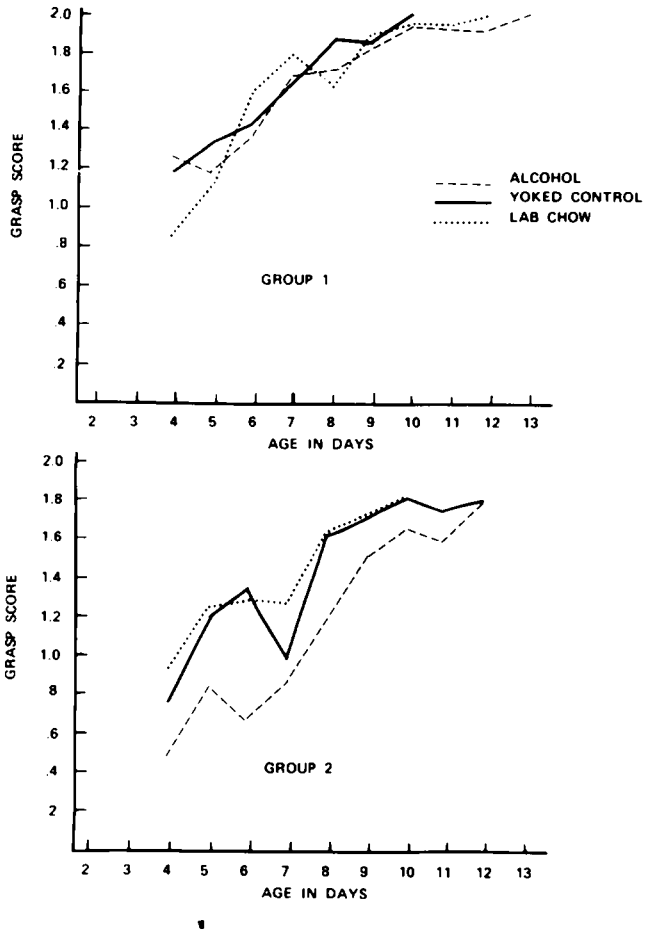

FIG. 3. Mean grasp scores of prenatal ethanol treatment and control mice as a function of postnatal age.

different from Group 1A. Control groups from the two treatment times did not differ from one another.

Figure 2 shows the mean weights for the pups over the 20 day testing period. A three factor (treatment type, treatment time, postnatal days) analysis of variance with repeated measures on one factor (postnatal days) of weights on postnatal days 5, 10, 15 and 20 resulted in a significant treatment type × treatment time × postnatal day interaction, $F(6,122)=2.93$, $p<0.025$. Post hoc analyses indicated that Group 1 showed very little variation (non-significant) among treatment groups at any of the four postnatal periods. In Group 2, differences were not significant on days 5 and 10. On days 15 and 20, Group 2A was significantly different from 2C and 2YC which did not differ from one another. Group 2A differed from Group 1A on day 20. Control groups did not differ from one another at any postnatal time.

Motor and sensory related developmental tests were initially analyzed using a two factor analysis of variance. The day of reaching criterion level was used as a dependent measure. This preliminary statistical approach revealed no differences between treatment types, treatment times or interaction of types × times. The measures were further analyzed using a three factor analysis of variance with repeated measures on one factor.

Motor Related Measures

With the exception of the expected significant alterations in scores of the motor related tests over testing days, the analyses of movement, righting, cliff aversion and vertical screen tests did not reveal any significant effects due to type or time of treatment or interactions among the factors. The development of these behaviors was unaffected by the experimental manipulations. Significant results were obtained from the analyses of the grasp reflex and horizontal screen tests. In the grasp reflex analysis, the time of treatment × testing day interaction was significant, $F(5,210)=2.74$, $p<0.025$. While this indicated that Group 2 was different from Group 1 on some portion of the testing days, an inspection of Fig. 3 suggested that the difference(s) was primarily due to the scores of Group 2A. Post hoc analyses indicated that Group 1 treatment groups did not differ from each other on any day, but that Group 2 animals were different on days 6, 7 and 8. On days 6 and 8, Group 2A was significantly different from Groups 2YC and 2C. On day 7, Group 2A differed from Group 2C. Group 2YC was not different from Group 2A or Group 2C. Group 2A differed from Group 1A on days 6, 7, and 8. Differences were not significant after day 8.

The horizontal screen analysis revealed a significant type

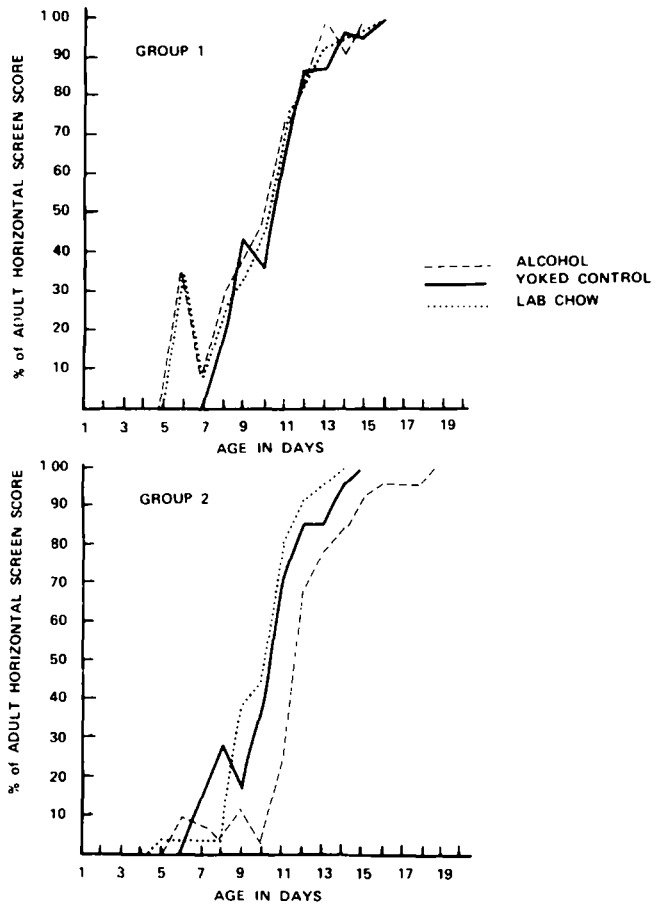

FIG. 4. Mean horizontal screen scores of prenatal ethanol treatment and control mice as a function of postnatal age.

of treatment × testing day interaction, $F(14,294)=2.27$, $p<0.01$. An inspection of Fig. 4 suggested that these results were due primarily to the low scores of Group 2A on several of the testing days. Post hoc analyses revealed that Group 1 animals showed similar developmental rates on this task. On day 9, Group 2C was significantly different from Groups 2A and 2YC which did not differ from one another. On days 10 and 11, Group 2A differed from Groups 2C and 2YC. Group 2A differed from Group 1A on all three days. Differences ceased to be significant after day 11.

Sensory Related Measures

No significant differences were found between groups for day of eye opening, ear opening, or presence of the auditory startle response.

Physical Malformations

No gross physical anomalies were anticipated because of the relatively low ethanol dosage and short duration of treatments. However, two out of five ethanol litters in Group 2 displayed some unusual characteristics on the day of birth. All of the pups in one litter had blood marked tail tips. The markings disappeared after about one week. Two of six pups in a second litter displayed a bloody mark under the skin on the nose or head area. These marks also disappeared within a few days and did not appear to be correlated with specific developmental problems. No other physical malformations were observed.

DISCUSSION

The results of this study suggest that exposure to non-teratogenic levels of ethanol during pregnancy can have pronounced effects on mouse offspring. The period of ethanol administration appears to be a critical factor in that markedly different effects are produced when ethanol treatment occurs during late pregnancy (Group 2) as compared to middle pregnancy (Group 1). The increase in neonatal deaths in the late gestation ethanol treatment group (Group 2A) is the most clearcut finding of the study. Group 2A animals were differentially affected by the drug treatment relative to their controls (Goups 2YC and 2C) while Groups 1A, 1YC, and 1C showed no difference in offspring viability.

Although these results seem to indicate that there is a critical period during gestation when ethanol can compromise neonatal survival, ethanol consumption factors may provide an explanation which is contrary to a purely critical period hypothesis. Differences in the quantity consumed and the pattern of intake of the ethanol containing liquid diet between Groups 1A and 2A dams may have resulted in a differential exposure to ethanol between Groups 1A and 2A fetuses. Group 2A fetuses may have been exposed to more ethanol than Group 1A fetuses. The experiment was designed to allow free access to the diets by the pregnant ethanol-treated mice so that consumption could be regulated by maintenance needs of the growing pups which require a greater consumption of nutrients as pregnancy progresses [39]. Had the intake for Group 2A been restricted to the Group 1A intake levels, the Group 2A mice would undoubtedly have been at a severe disadvantage in trying to meet the increasing needs of pregnancy.

Although Group 2A fetuses may have received more ethanol than their Group 1 counterparts it is possible that the former had a greater metabolic capacity than the latter. Fetal rat liver aldehyde dehydrogenase (ALDH) has been found to be active beginning on day 15 of gestation, with a small, but rapid increase at birth [47]. If the alcohol metabolite acetaldehyde mediates some of the toxic effects of ethanol on fetal development [37], Group 2A may have been exposed to less of the metabolite than was Group 1A since ALDH catalyzes acetaldehyde oxidation. On the other hand, the mother's placenta may act as an auxiliary liver during early pregnancy as the fetal liver is developing since placental ALDH activity decreases in proportion to increasing fetal liver enzyme activity [47]. The complexity of these alternative hypotheses concerning ethanol effects on Groups 1A and 2A combined with the fact that the present investigation was not designed to determine directly the amount of fetal ethanol exposure offer little basis for concluding whether Group 1A or 2A fetuses received greater, lesser or equal prenatal exposure to ethanol and/or its metabolites. Further research should incorporate measures of ethanol and acetaldehyde levels in the fetus during the two prenatal periods. The different consumption patterns during these periods also need further investigation. This additional information is necessary in order to determine whether the period of gestation, rather than the amount of ethanol consumed by the

dam, and/or her consumption pattern is critical to the decreased neonatal survival observed in the present study.

Several previous reports have noted increases in neonatal deaths after prenatal exposure to ethanol. Children of chronic alcoholic women are prone to increased neonatal mortality [19, 27, 29, 36], but critical periods for ethanol consumption have not been determined. Long term ethanol administration (both before and throughout pregnancy) resulted in increased mortality of infant rats during the first three post-natal days, the only period analyzed [17]. Ethanol treatment throughout pregnancy resulted in decreased viability of rat offspring during the pre-weaning period [3,31]. Exposure of the long sleep and short sleep lines of mice to ethanol during gestation days 1–10, 11–20 or 1–20 resulted in 50% neonatal deaths of long sleep offspring treated during the latter two time periods [52]. Similarly, offspring of C57BL mice administered ethanol during the second half of pregnancy exhibited significantly more neonatal deaths than did those exposed during the first half of gestation (Fish, in preparation). The present study indicates a more restricted susceptible period for prenatal ethanol-induced deaths in heterogenous stock (HS) mice.

The use of both pair fed and lab chow fed controls is the most effective way to estimate the extent to which group differences are due to the effects of malnutrition (the difference between Groups C and YC scores) or to the ethanol itself (the difference between Groups A and YC scores). The non-differential weight gains across all groups of pregnant dams would seem to indicate no obvious detrimental effect due to the substitution of the liquid diets for a short duration. However, maternal weights may not be a valid index of fetal well-being since the nutritional component seemed to be influential in determining birthweight. In Group 2A birthweights were significantly depressed in comparison to Group 1C, 2C and 1A birthweights while Groups 1YC and 2YC weights were not significantly different from any of the other group birthweights. This indicates that the lowered birthweights following the late administration of the ethanol containing liquid diet are partially due to the alcohol itself and partly due to nutritional deficits.

Maternal alcoholism during pregnancy is generally associated with decreased birthweight in humans [19]. Moderate ethanol consumption during late pregnancy resulted in decreased birthweights not seen after early pregnancy consumption [28]. Previous animal research yielded conflicting results concerning ethanol-induced birthweight alteration. No differences between ethanol treated offspring and their controls [32, 35, 59] or lowered birthweights for ethanol treated animals [4,15] have been noted. Decreased birthweights of both ethanol and sucrose control treated groups have also been described [1, 31, 56].

After fostering of the neonates to surrogate mothers, Group 2A weights exhibited a temporary "catch up" reflected in the analysis of weights on postnatal days 5 and 10. Although the weights of all of the groups increased throughout the neonatal period, Group 2A weights deviated from all of the others on days 15 and 20. The reason(s) for the initial "catch up" followed by the slower growth of Group 2A in comparison to the other groups is not obvious. The untreated surrogate mothers might have had a short-term, positive influence on Group 2A. Those factors associated with increasing growth after the 10th postnatal day might have been critically altered by the late prenatal ethanol treatment.

Behavioral measures were relatively unaffected by the prenatal treatments. The time required to reach adult performance levels did not differentiate any group on any task. Developmental delays, expressed by qualitative changes in the rates of development of adult proficiency, were observed in the grasp and horizontal screen tasks. These delays, occuring between days 6 to 11, were transitory and were found primarily in Group 2A animals. On two occasions (Grasp Reflex—Day 7 and Horizontal Screen Task—Day 9) the low scores of Group 2YC suggest a possible contribution of nutritional factors to the impairments of Group 2A. It should be noted that both tasks require forelimb manipulative capacities which may be altered by the late gestation alcohol treatment. The ontogeny of many of the same behaviors determined in the present study, often quantified as day of response appearance or studied at one postnatal time, was not different between treatment groups in other studies [13, 31, 59] although differences on some behaviors have been reported [13,31]. The nutritional component was not implicated.

Sensory related measures did not appear to be affected by ethanol exposure, a result which does not correspond with some earlier findings [22, 31, 59]. These researchers, however, administered higher levels of ethanol than in the present study and/or utilized different strains of animals without pair fed controls or cross-fostering.

It is possible that more differences in the measured indices might have been observed if all animals had lived to be tested. The high frequency of deaths among Group 2A may have eliminated the lower end of the score distribution for this group. Greater differences might also have been observed if the conservative means of selecting which experimental animals to cross-foster had been a random choice. Instead, the healthiest animals were chosen in an effort to increase survival chances throughout the daily testing. Both of these factors (neonatal deaths and cross-fostering choices) may have systematically eliminated an important portion of the sample.

Overall, the level and duration of ethanol exposure in the present study allows for relatively normal sensorimotor development when administered late in gestation and has no observable effect when administered during the middle of gestation. The intact development of the offspring treated during the mid gestational period—the earliest period of brain development—allows for the further investigation of learning and memory capacities of these mice in the absence of the confounds of sensorimotor deficits. Further research is necessary to determine the causative factors involved in the severe decrease in offspring viability, the lower birthweight and the slower growth during the neonatal period of the animals exposed to ethanol late in gestation.

REFERENCES

1. Abel, E. L. Effects of ethanol on pregnant rats and their offspring. *Psychopharmacology* **57**: 5–11, 1978.
2. Abel, E. L. Prenatal effects of alcohol on adult learning in rats. *Pharmac. Biochem. Behav.* **10**: 239–243, 1979.
3. Abel, E. L. and B. A. Dintcheff. Effects of prenatal alcohol exposure on growth and development in rats. *J. Pharmac. exp. Ther.* **207**: 916–921, 1978.

4. Anandam, N., W. Felegi and J. Stern. Fetal alcohol enhances auditory startle in rats. Paper presented at *Int. Soc. Devl Psychobiol.*, Atlanta, November, 1977.
5. Angevine, J. B., Jr. Critical cellular events in the shaping of neural centers. In: *The Neurosciences. Second Study Program*, edited by F. O. Schmidt. New York: The Rockefeller University Press, 1970, pp. 62–72.
6. Baer, D. S. and D. W. Crumpacker. Effects of maternal ingestion of alcohol on maternal care and behavior of progeny in mice. *Behav. Genet.* **5:** 88–89, 1975.
7. Beyers, N. and A. Moosa. The fetal alcohol syndrome. *S. Afr. Med. J.* **54:** 575–578, 1978.
8. Bond, N. W. and E. L. Di Giusto. Effects of prenatal alcohol consumption on open-field behavior and alcohol preference in rats. *Psychopharmacology* **46:** 163–165, 1976.
9. Bond, N. W. and E. L. Di Giusto. Avoidance conditioning and Hebb-Williams maze performance in rats treated prenatally with alcohol. *Psychopharmacology* **58:** 69–71, 1978.
10. Branchey, L. and A. J. Friedhoff. Biochemical and behavioral changes in rats exposed to ethanol *in utero*. *Ann. N.Y. Acad. Sci.* **273:** 328–330, 1976.
11. Chernoff, G. F. The fetal alcohol syndrome in mice: an animal model. *Teratology* **15:** 223–230, 1977.
12. Clarren, S. K., E. C. Alvord, Jr., I. M. Sumi, A. P. Streissguth and D. W. Smith. Brain malformations related to prenatal exposure to ethanol. *J. Pediat.* **92:** 64–67, 1978.
13. Demers, M. and G. Kirouac. Prenatal effects of ethanol on the behavioral development of the rat. *Physiol. Psychol.* **6:** 517–520, 1978.
14. Druse, M. J. and J. H. Hofteig. The effect of chronic maternal alcohol consumption on the development of central nervous system myelin subfractions in rat offspring. *Drug Alcohol Depend.* **2:** 421–429, 1977.
15. Fernandez, K., W. F. Caul, G. L. Osborne and G. I. Henderson. Effects of maternal chronic ethanol administration on offspring open-field and y-maze performance. Paper presented at *Int. Soc. Devl Psychobiol.*, Atlanta, November, 1977.
16. Fox, M. W. Reflex ontogeny and behavioral development of the mouse. *Anim. Behav.* **13:** 234–241, 1965.
17. Henderson, G. I. and S. Schenker. The effect of maternal alcohol consumption on the viability and visceral development of the newborn rat. *Res. Communs chem. pathol. Pharmac.* **16:** 15–32, 1977.
18. Hutchings, D. E. Behavioral teratology: embryopathic and behavioral effects of drugs during pregnancy. In: *Early Influences*, edited by G. Gottlieb. New York: Academic Press, 1978, pp. 7–34.
19. Jones, K. L. The fetal alcohol syndrome. *Addict. Dis.* **2:** 79–88, 1975.
20. Jones, K. L. and D. W. Smith. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* **2:** 999–1001, 1973.
21. Kaminski, M., C. Rumeau-Roquett and D. Schwartz. Consommation d'alcohol chez les femmes encientes et issue de la grossesse. *Rev. Epidemiol. Sante Publique* **24:** 27–40, 1976.
22. Kronick, J. B. Teratogenic effect of ethyl alcohol administered to pregnant mice. *Am. J. Obstet. Gynec.* **124:** 647, 1976.
23. Krsiak, M., J. Elis, N. Poschlova and K. Masek. Increased aggressiveness and lower brain serotonin levels in offspring of mice given alcohol during gestation. *J. Stud. Alcohol* **38:** 1696–1704, 1977.
24. Kumar, S. and A. K. Rawat. Prolonged ethanol consumption by pregnant and lactating rats and its effects on cerebral dopamine in the fetus and neonate. *Res. Communs Psychol. Psychiat. Behav.* **2:** 259–278, 1977.
25. Landesman-Dwyer, S., S. Keller and A. P. Streissguth. Naturalistic observations of newborns: effects of maternal alcohol intake. *Alcohol: Clin. exp. Res.* **2:** 171–177, 1978.
26. Lanier, L. P., A. J. Dunn and C. VanHartesveldt. Development of neurotransmitters and their function in brain. *Rev. Neurosci.* **2:** 195–256, 1976.
27. Lemoine, P., H. Haronsseau, J. P. Borteryu and J. C. Menuet. Les enfants de parents alcooliques: Anomalies observees a propos de 127 cas. *Ouest. Med.* **25:** 476–482, 1968.
28. Little, K. Moderate alcohol use during pregnancy and decreased infant birth weight. *Am. J. Publ. Hlth* **67:** 1154–1156, 1977.
29. Majewski, F., J. Bierich, H. Loeser, R. Michaelis, B. Leiber and F. Bettecker. Zur klinik und pathogenese der alkoholembryopthie. Bericht uber 68 falle. *Munch. Med. Wosch.* **118:** 1635–1642, 1976.
30. Martin, J. C., D. C. Martin, B. Radow and G. Sigman. Blood alcohol levels and caloric intake in the gravid rat as a function of diurnal period, trimester, and vehicle. *Pharmac. Biochem. Behav.* **8:** 421–427, 1978.
31. Martin, J. C., D. C. Martin, G. Sigman and B. Radow. Offspring survival, development and operant performance following maternal ethanol consumption. *Devl Psychobiol.* **10:** 435–446, 1977.
32. Martin, J. C., D. C. Martin, G. Sigman and B. Radow. Maternal ethanol consumption and hyperactivity in cross-fostered offspring. *Physiol. Psychol.* **6:** 362–365, 1978.
33. McClearn, G. E., J. R. Wilson and W. Meredith. The use of isogenic and heterogenic mouse stocks in behavioral research. In: *Contributions to Behavioral-Genetic Analysis: the Mouse as a Prototype*, edited by G. Lindsey and D. D. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 3–22.
34. Mulvihill, J. J. and A. M. Yeager. Fetal alcohol syndrome. *Teratology* **13:** 345–348, 1976.
35. Oisund, J. F., A.-E. Fjorden and J. Morland. Is moderate ethanol consumption teratogenic in the rat? *Acta pharmac. tox.* **13:** 345–348, 1976.
36. Olegard, R., K. G. Sabel, M. Kyllerman, B. Sandin, M. Aronsson, P. R. Johansson and C. Carlsson. Barn till kvinnliga alkoholister-en inventering au 15 syskonskaror. *Acta. Soc. Med. Suec.* **84:** 424, 1975.
37. O'Shea, K. S. and M. H. Kaufman. The teratogenic effect of acetaldehyde: implications for the study of the fetal alcohol syndrome. *J. Anat.* **128:** 65–76, 1979.
38. Oulette, E. M., H. L. Rosett, N. P. Rosman and L. Weiner. Adverse effects on offspring of maternal alcohol abuse during pregnancy. *New Engl. J. Med.* **297:** 528–530, 1977.
39. Poulous, B. K. The effect of ethanol on plasmalogen levels in selected areas of the adult and developing C57BL/6J mouse brain. Unpublished masters thesis, University of Colorado, Department of Pharmacology, School of Medicine, 1977.
40. Randall, C. L. and W. J. Taylor. Prenatal ethanol exposure in mice: teratogenic effects. *Teratology* **19:** 305–312, 1979.
41. Randall, C. L., W. J. Taylor and D. W. Walker. Ethanol-induced malformations in mice. *Alcoholism: Clin. exp. Res.* **1:** 219–224, 1977.
42. Rawat, A. K. Ribosomal protein synthesis in the fetal and neonatal rat brain as influenced by maternal ethanol consumption. *Res. Communs chem. pathol. Pharmac.* **12:** 723–732, 1975.
43. Rawat, A. K. Effects of maternal ethanol consumption on the fetal and neonatal cerebral neurotransmitters. In: *The Role of Acetaldehyde in the Actions of Ethanol*, edited by K. O. Lindros and C. J. P. Eriksson. Helsinki, Finland: The Finnish Foundation for Alcohol Studies, 1975, pp. 158–176.
44. Riley, E. P., E. A. Lochry and N. R. Shapiro. Lack of response inhibition in rats prenatally exposed to alcohol. *Psychopharmacology* **62:** 47–52, 1979.
45. Riley, E. P., E. A. Lochry, N. R. Shapiro and J. Baldwin. Response perseveration in rats exposed to alcohol prenatally. *Pharmac. Biochem. Behav.* **10:** 255–259, 1979.
46. Riley, E. P., N. R. Shapiro and E. A. Lochry. Nosepoking and head dipping behaviors in rats prenatally exposed to alcohol. *Pharmac. Biochem. Behav.* **11:** 513–519, 1979.
47. Sjoblom, M., L. Pilstrom and J. Morland. Activity of alcohol dehydrogenase and acetaldehyde dehydrogenases in the liver and placenta during development of the rat. *Enzyme* **28:** 108–115, 1978.
48. Streissguth, A. P. Physiologic handicaps in children with the fetal alcohol syndrome. *Ann. N.Y. Acad. Sci.* **273:** 140–145, 1976.

49. Streissguth, A. P. and H. M. Barr. Brazelton assessment of neonatal offspring of moderate and light drinkers. Paper presented at Society for Research in Child Development, New Orleans, March, 1977.
50. Streissguth, A. P., C. S. Herman and D. W. Smith. Stability of intelligence in the fetal alcohol syndrome: a preliminary report. *Alcohol: Clin. exp. Res.* **2**: 165–170, 1978.
51. Streissguth, A. P., J. C. Martin and D. C. Martin. Research design and assessment of alcohol consumption during pregnancy. Paper presented at Society for Research in Child Development, New Orleans, March, 1977.
52. Swanberg, K. M. and D. W. Crumpacker. Genetic differences in reproductive fitness and offspring viability in mice exposed to alcohol during gestation. *Behav. Biol.* **20**: 122–127, 1977.
53. Thadani, P. V., C. Lau, T. A. Slotkin and S. M. Schanberg. Effects of maternal ethanol ingestion on amine uptake into synaptosomes of fetal and neonatal rat brain. *J. Pharmac. exp. Ther.* **200**: 292–297, 1977.
54. Tze, W. J. and M. Lee. Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. *Nature* **257**: 479–480, 1975.
55. Wahlsten, D. A developmental time scale for postnatal changes in brain and behavior of B6D2F2 mice. *Brain Res.* **72**: 251–264, 1974.
56. Weiner, S. G., W. Shoemaker and F. E. Bloom. Interaction of alcohol and nutrition during gestation: influence on maternal and offspring development in the rat. Paper presented at *Int. Soc. Devl Psychobiol.*, Atlanta, November, 1977.
57. Yanai, J. and B. E. Ginsburg. The effect of alcohol consumed by parent mice on the susceptibility to audiogenic seizure and the open-field behavior of their offspring. *Behav. Genet.* **3**: 418, 1973.
58. Yanai, J. and B. E. Ginsburg. Long term effect of early ethanol on predatory behavior in inbred mice. *Physiol. Psychol.* **4**: 409–411, 1976.
59. Yanai, J. and B. E. Ginsburg. A developmental study of ethanol effect on behavior and physical development in mice. *Alcoholism: Clin. exp. Res.* **1**: 325–333, 1977.