Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 331–336, 1983.

Neonatal Ethanol Exposure: Effects on Adult Behavior and Brain Growth Parameters[1]

KATHLEEN A. GRANT,[2] ELIZABETH Y. CHOI AND HERMAN H. SAMSON[3]

Department of Psychology, University of Washington, Seattle, WA 98195

GRANT, K. A., E. Y. CHOI AND H. H. SAMSON. *Neonatal ethanol exposure: Effects on adult behavior and brain growth parameters.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 331–336, 1983.—Neonatal rats were reared using an artificial feeding technique from postnatal day 4 through 18. On postnatal day 4 through 7, some animals were given ethanol in their milk formula with the remaining animals serving as controls. The ethanol was given in amounts that have been shown to induce microcephaly when animals are examined at 18 days after birth. In this study, on postnatal day 18, all animals were weaned and allowed ad lib food and water until they were sacrificed at 60 days of age. When the animals were 30 days old, they were tested on a battery of behavioral tasks (nose poke, passive avoidance, and open field). No differences were found between the ethanol exposed animals and their controls on passive avoidance or nose poke activity. Ethanol-exposed female animals showed increased activity compared to their controls in the open field. There were no differences in open field activity between the ethanol exposed males and their controls. An examination of brain growth parameters (wet weights, DNA, cholesterol and protein content) showed no difference between the brains of ethanol-exposed males compared to controls at 60 days of age, regardless of brain parameter or brain area studied (forebrain, cerebellum or brainstem). The brains of ethanol-exposed females, however, had considerably less catch-up growth, with the ethanol effect on the cerebellum being very similar to that observed at 18 days of age. The results imply that sex and the time of ethanol exposure may interact to determine the ability of the brain to develop following a neonatal alcohol insult.

Neonatal ethanol exposure Brain growth parameters Behavioral effects Fetal alcohol syndrome (FAS) Sex differences

THE Fetal Alcohol Syndrome (FAS) consists of a constellation of abnormal developmental characteristics found in children whose mothers ingest large amounts of ethanol during pregnancy [16, 37, 38]. The major features of FAS include general growth retardation, facial dysmorphology, and Central Nervous System (CNS) deficits. One striking feature in FAS is microcephaly as measured by a head circumference greater than two standard deviations below normal [27]. Behavioral impairments found in FAS children, including learning disabilities, hyperactivity, low IQ scores, and impairment in motor coordination, also reflect possible CNS deficits [35].

It has been established in many different animal models that deficits in development result when ethanol is administered either in utero or early postnatal life. (For a review see [2]). In our laboratory, we have been primarily concerned with the effects of ethanol administration to the neonatal rat on brain development and behavior [19, 23, 31, 32, 33]. Although it is well established that ethanol can interfere with the development of many organ systems if it is administered at various times throughout gestation [3, 28, 36], the CNS is specifically vulnerable to ethanol's effects during the late prenatal and early postnatal period [9, 10, 14, 19]. At this time the brain undergoes a growth spurt characterized by extensive cellular proliferation, differentiation, gliogenesis, and synaptogenesis [5, 6, 20]. A dramatic impairment of brain growth was demonstrated when neonatal rats were administered moderately high doses of ethanol for four days during part of their brain growth spurt (postnatal days 6 through 9; [19]). The nature of this microcephaly observed at 18 days of age has been partially characterized, with decreases in biochemical indicies of cellularity, myelination/arborization, and cell size being evident [23].

In the present study, three additional questions concerning this ethanol induced microcephaly were addressed. First, are any behavioral deficits associated with it? Second, does the microcephaly, apparent ten days after alcohol administration (postnatal day 18), persist into adulthood (postnatal day 60)? Finally, if microcephaly does persist into adulthood, is the biochemical profile of the ethanol-exposed microcephalic brains at 60 days similar to that found at 18 days of age?

[1]Supported in part by funds from the Alcoholism and Drug Abuse Institute of the University of Washington, and in part by funds from the Distilled Spirits Council of the U.S. Inc.

[2]Supported by a NSF Predoctoral Fellowship. Requests for reprints should be addressed to K. A. Grant, Department of Psychology NI-25, University of Washington, Seattle, WA 98125.

[3]Supported by a National Institute on Alcohol Abuse and Alcoholism Career Development Award (KO2 AA 00066).

METHOD

Breeding

Ninety day old female rats (Long Evans strain) were obtained from the University of Washington vivarium, housed in standard rodent hanging cages, and given ad lib food (Purina Lab Chow) and water. Room temperature was controlled at 23°C with artificial lighting maintained on a twelve hour on/off cycle (on at 8:00 am). After the females had habituated to the new housing condition (approximately 2–3 days) they were placed individually into breeding cages with experienced male breeders (Long Evans strain). Twice daily, pans below the wire mesh floor of the breeding cages were examined for the presence of copulatory plugs. When plugs were detected, the female was removed, weighed and placed in a solid floor nesting cage with wood shavings available for nesting material. The day of plug detection was assigned as Day 0 and all references to gestational age are relative to this day.

Twenty four hours after parturition, litters were culled to 8 pups. When possible, the 4 heaviest males and 4 heaviest females were kept. The neonates remained with their dam until Day 26 (postnatal day 6), when they received a gastric catheter. Five litters were used for these experiments with each litter divided into control and ethanol exposed groups.

Surgery, Incubation, Feeding and Maintenance

The entire artificial rearing procedure, including catheter construction and surgical technique has been described in detail [32] and will not be elaborated upon here. Briefly, the animal is implanted with a gastric catheter and then placed individually into a weighted plastic cup which floats in a 37°C water bath. The catheter is connected to a syringe containing the milk formula (see [32]). The syringe is placed in an infusion pump (8 syringes/pump; Harvard Apparatus Corp., series 935) controlled by programmable timing equipment which provides a 20 minute feed every 2 hours. The amount of milk formula delivered increases each day to match the weight gains of the Dam reared pups. At the same time each day the animals were disconnected from the syringes, weighed and their catheters adjusted to accommodate growth. The syringes were washed and filled with fresh milk. Twice a day the animals were stroked lightly in the anal/genital area to stimulate urination and defecation. The emergence of developmental milestones, such as tooth eruption, eye opening and ear development were checked for daily.

Ethanol Exposure

Following the catheter placement, the animals were divided into two groups: 3 animals received ethanol in their milk formula and 3 animals received only the milk formula. The ethanol was administered on four days (Day 26, 27, 28 and 29) and was given in doses that had previously been shown to result in microcephaly using this artificial rearing technique [19,23].

Behavioral Testing

On Day 40 (postnatal day 18), the animals were removed from the artificial rearing condition and their catheters cut next to the abdominal wall and heat sealed. Each litter was then housed together and weaned on milk formula mixed with powdered rat chow and water. They remained housed together until postnatal day 30, at which time they were given a battery of behavioral tests including open field activity, nose poke and passive avoidance (see below). All testing was done in the middle of the day, during the light portion of the day/night cycle.

Open field activity. The open field had a diameter of 36 inches and was divided into an inner circle (diameter of 12 inches) and two outer rings, both having a 6 inch width. The field was divided into 9 sections; the inner circle, the middle ring divided into three equal sections, and the outer ring divided into 5 equal sections. Each animal was given one, three-minute trial. The rat pup was placed in the center of the open field with a plastic cup over it. The trial began when the cup was removed. Five measures were taken: latency to cross out of the inner circle, time spent in the 4 centermost sections, the number of sections crossed into, the number of rearings (two front legs off the ground not touching the sides of the field and not including grooming postures), and the number of defecations.

Nose Poke

The nose poke apparatus was a black Plexiglas box (12×12×8 inches) with a hole drilled 2 inches from the bottom of one side. A poke was recorded when the animal's snout extended through the hole and could be visualized by an observer perpendicular to the plane of the box containing the hole. Two measures were recorded: latency to first poke and the number of pokes in a five minute trial.

Passive Avoidance

The passive avoidance task was run in a 22×5 inch Plexiglas box which was divided in half by a sliding partition. Half of the chamber was painted black with a black lid and the other half was painted white with a transparent lid. The floor of the box was composed of quarter inch rods which were wired to conduct electrical shock. One half of the chamber was designated as the "unsafe" side. For some animals the white side was the "unsafe" side, and for others the black side was "unsafe". The animal was placed in the "safe" side and the partition separating sides lifted. If the animal completely entered the "unsafe" side, a 5 m Amp shock was given until it returned to the "safe" side. Each trial lasted up to three minutes with a successful trial one in which the animal avoided shock by not entering the "unsafe" side for the full three minutes. If the animal failed to avoid the "unsafe" side, the trial was terminated and shock was given. Five successful trials out of seven completed the test. Three measures were recorded: latency to first shock, trials to second shock and the number of trials required to reach the 5 of 7 criteria.

Following behavioral testing, the animals were housed individually with ad lib chow and water. They were weighed daily and checked for any obvious signs of failure to thrive.

Brain Dissection

When the animals were 60 postnatal days old, they were weighed, decapitated and their brains removed. The olfactory bulbs were removed and the remaining brain cut transversely at the brainstem just rostral to the medulla oblongata. A wet weight of the whole brain was taken using a Mettler A 30 scale. Then the brain was dissected transcollicularly, and the cerebellum separated from the brainstem by dissection at the peduncles. These cuts left the brain in three sections; the forebrain (telencephalon and di-

TABLE 1

PERFORMANCE ON BEHAVIORAL TESTS SEPARATED BY SEX AND GROUP†

Task	Nose Poke		Passive Avoidance			Open Field				
Group	Number of Pokes	Latency to 1st Poke	Trials to Criteria	Latency to 1st Shock	Trails to 2nd Shock	Crosses	Rears	Defecations	Latency to Leave Center	Total Time in Center
Males										
Control	19	17.6	7	19.9	2	22	17	3	14.2	31.4
(n=7)	(9–41)	(14.8)	(6–8)	(10.3)	(2–7)	(15–34)	(6–34)	(0–5)	(7.9)	(17.8)
Ethanol	15.5	20.1	7.5	13.5	4	20	13	2	25.0	54.7
(n=6)	(10–21)	(21.4)	(7–11)	(9.8)	(2–7)	(6–33)	(4–18)	(0–4)	(16.4)	(31.9)
Females										
Control	19	39.9	8	13.3	2	20	14	5	15.4	42.9
(n=5)	(6–23)	(25.3)	(6–9)	(5.8)	(2–7)	(12–31)	(5–32)	(0–6)	(6.7)	(22.3)
Ethanol	21	6.5	6	32.8	5	47.5*	13	4.5	12.2	89.8*
(n=4)	(9–27)	(16.7)	(6–8)	(41.2)	(2–7)	(38–50)	(12–19)	(0–7)	(2.6)	(33.7)

*Significantly different from same sex control at $p<0.05$.

†Data for latency and time scores in seconds are means (±SD); All other scores are medians (range).

encephalon), the cerebellum and the brainstem. The forebrain was sectioned bilaterally and one half was weighed and frozen until assayed. Similarly, both the cerebellum and brainstem were weighed and frozen. In addition to brain weights, the heart, liver, kidney and adrenal glands were removed and wet weights taken.

Biochemical Analysis

The frozen tissue samples were thawed at room temperature and the DNA, protein and cholesterol extracted according to the procedure previously used in our lab [23]. Briefly, the procedure used to measure DNA was a modification of the diphenylamine colorimetric method described in Zamenhof *et al.* [40]. Cholesterol was assayed using the colorimetric analysis of Searcy and Berquist [34]. Total protein was determined by the method of Lowry *et al.* [26].

The DNA content was taken to represent the number of cells present in the sample. The amount of cholesterol was taken to be representative of the membrane content of the sample (thus myelination and arborization of the tissue). Total protein in the samples was used as an index of general growth.

Statistical analysis. Comparisons between groups were made by one way analysis of variance [22] or Mann Whitney U [30], with $p<0.05$ required for significance.

RESULTS

Of the 30 animals artificially reared, 22 completed the experiment. Eight animals died within the first week from surgical complications.

The mean±SD ethanol doses for the four days of exposure were: Day 26=3.7 g/kg±0.4; Day 27=7.4 g/kg±0.7; Day 28=1.6 g/kg±0.2; and Day 29=9.2 g/kg±1.4. While receiving the alcohol, all ethanol animals showed behavioral signs of intoxication (most notable was a highly impaired righting reflex). Twenty-four to thirty-six hours after the alcohol-containing milk had been replaced with the milk formula only, the ethanol-treated animals showed signs of ethanol withdrawal, including full body tremors and extreme head bobbing activity.

There was no difference between groups with respect to tooth emergence, eye opening and outer ear development. A sex difference in weight gain was noticeable from postnatal day 21 to the end of the experiment; however, there were no weight differences between ethanol-treated and control animals with sex (Males at 60 days of age: ethanol-treated=281 g±23, control=278 g±38; Females at 60 days of age: ethanol-treated=189 g±15, control=185 g±12 (mean±SD)). Heart, liver, kidney and adrenal weights were not different for any condition within each sex.

Table 1 summarizes the results of the behavioral tests. There were no significant differences in nose poke or passive avoidance behavior among groups. Similarly, the alcohol-treated and control males did not differ from one another in the open field test. The females, however, did show a group difference on the open field. The ethanol-exposed females made more total crosses and spent more time in the center of the field. There were no significant differences, however, with respect to the number of rears, defecations or latency to leave the center section.

The brain/body weight ratios, forebrain and hindbrain weights for the animals are presented in Fig. 1. For comparison purposes, the brain weight parameters from a previous study [23] are given for eighteen day old animals that received identical ethanol treatment. At postnatal day 18 no sex differences in the brain parameters were found and the male and female data have been combined. At 60 days of age, the ethanol-treated males had a brain/body weight ratio similar to their controls (see Fig. 1). The reduction in the degree of microcephaly previously found at 18 days of age represents a significant "catch-up" in the brain weight of this group, from a 20% reduction (3.1 SD below the control mean [32]) at 18 days to a 5% reduction (1.5 SD below the control mean) at 60 days. The ethanol-exposed females at 60 days of age continued to have microcephaly compared to their controls, although the degree of microcephaly had been reduced from a 20% decrease (3.1 SD below the control mean [32]) at 18 days to a 12% decrease (3.3 SD below the control mean) at 60 days.

The forebrain sample weights of the ethanol-exposed

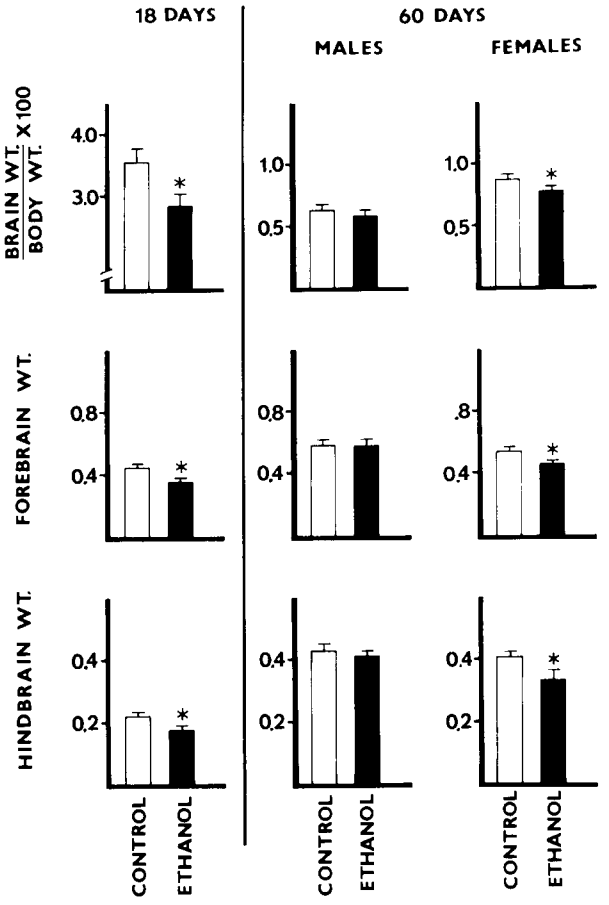

FIG. 1. Brain weights (g) for 18-day-old and 60-day-old ethanol-treated and control animals. (Mean±SD, *=Significant from control with $p<0.05$).

males at 60 days were identical to their controls. The forebrain samples of the ethanol-treated females did not "catch up" with the control female forebrain samples and still remained significantly lighter at 60 days of age ($p=0.05$), although the weight difference had decreased from 20% to 13%. The hindbrain (cerebellum plus brainstem) weights for the ethanol-exposed males were also not different from those of their controls at 60 days of age. In contrast the ethanol-treated females had a deficit in hindbrain weight at 60 days that was very similar to that noted at 18 days (18% and 19% respectively).

The biochemical profiles of the brains are given in Table 2. Again for comparison the biochemical values for 18-day-old animals are included. The brains of control and ethanol-exposed males did not differ from one another in the content of DNA, cholesterol or protein in any of the brain areas examined. In contrast, the females differed in the amount of DNA and cholesterol in the cerebellum, with the brains of the ethanol-exposed animals containing lower amounts. As well, the brains of the ethanol-exposed females contained lower amounts of DNA, cholesterol and protein in the forebrain samples, although these differences were not significant.

DISCUSSION

The effects of postnatal ethanol exposure found in this study depended on the behavioral task examined, the brain parameter measured and the sex of the animal. These ethanol effects would appear to be independent of nutritional influences since the ethanol-treated and the control groups did not differ in body weight. In addition, at the time of sacrifice, no effects of alcohol on heart, liver or kidney weights were found.

No systematic effects of ethanol exposure were found on behavioral measures generally taken as "exploratory behavior" or "emotionality" [1, 4, 12, 15, 29]. The open field test has been a standard procedure used to assess the behavioral effects of early ethanol exposure. Investigators have found no difference in behavior [4], increases in ambulation [12,15], decreases in rearing [1], and age-dependent changes in activity [12] in animals exposed to ethanol *in utero*. In this study, there were no differences between males exposed to ethanol and their controls in any of the open field measures taken. The ethanol-exposed females, however, showed increased ambulation compared to their controls. In addition to the increase in the number of sections crossed, the time spent in the central portion of the field by the ethanol females was atypical, as the female controls and the males of both groups spent more time around the periphery of the maze.

As presented in Table 1, no significant differences were found in the nose poke test or the passive avoidance learning task for any condition. These results are different from the effects of *in utero* exposure to ethanol. Riley *et al.* [29] have reported increases in the number of nose pokes and a decrease in latency to first poke for 29-day-old male rats. Also, an increase in the number of trials to criteria for a passive avoidance study in 22-day-old rats (not separated by sex) has been reported [25]. In both of these studies the animals were exposed to ethanol from gestation Day 5 through 20. Reexamining our data with the criteria for passive avoidance learning specified by Lochery and Riley [25], we still failed to find any significant difference between the ethanol-exposed pups and their controls. These conflicting results may be due to differences in the time of ethanol exposure, the dose of ethanol or the age of the animals at testing. The difference in relative preference for the center of the field, plus the lack of a difference in either the nose poke activity or passive avoidance behavior suggests that the altered behavior of the ethanol-exposed females can not be explained by only a general increase in activity, or by a general lack of response inhibition.

The biochemical parameters measured in brains of 18-day-old rats, compared to 60-day-old rats given the same ethanol treatment, indicate that substantial growth occurred for all conditions during that time period. On all measures of brain growth (wet weight, DNA, cholesterol, or protein content), in all areas examined (forebrain, cerebellum, and brainstem), the brains of ethanol-exposed males were indistinguishable from those of their controls at 60 days of age (Fig. 1, Table 2). In contrast, the brains of ethanol-exposed females continued to show microcephaly and decreased forebrain and hindbrain weights. It is interesting to note that although the forebrain samples of ethanol-exposed females were weight-deficient at 60 days, there was perhaps a partial "catch-up" growth. Unlike the decreased cholesterol/DNA and protein/DNA ratios seen in 18-day-old ethanol-exposed animals [23], at 60 days these ratios were not affected by ethanol treatment in either males or females. The hindbrains of ethanol-exposed females, however, had nearly the identical percentage weight deficit at 60 days of age that was observed at 18 days, indicating very little catch-up growth

TABLE 2

MEAN±SD OF BIOCHEMICAL PARAMETERS

	Tissue Wt. (g)	DNA (mg)	Cholesterol (mg)	Protein (mg)
		18 Day Old		
Forebrain				
Control (n=8)	0.442 ± 0.024	0.453 ± 0.003	3.26 ± 0.53	26.4 ± 2.96
Ethanol (n=6)	0.356 ± 0.025*	0.459 ± 0.029	2.60 ± 0.46*	22.4 ± 1.93*
Hindbrain				
Control (n=8)	0.220 ± 0.016	1.165 ± 0.117	2.07 ± 0.37	12.6 ± 0.70
Ethanol (n=6)	0.178 ± 0.020*	0.991 ± 0.134*	1.51 ± 0.14*	10.5 ± 1.35*
		60 Day Old		
Forebrain				
Males				
Control (n=5)	0.583 ± 0.032	0.619 ± 0.035	7.28 ± 0.58	36.6 ± 5.85
Ethanol (n=5)	0.584 ± 0.015	0.592 ± 0.077	7.28 ± 0.14	36.9 ± 3.64
Females				
Control (n=4)	0.542 ± 0.013	0.602 ± 0.062	7.01 ± 0.61	36.2 ± 3.87
Ethanol (n=4)	0.473 ± 0.005*	0.519 ± 0.096	6.35 ± 0.80	31.5 ± 2.52
Brainstem				
Males				
Control (n=6)	0.208 ± 0.021	0.226 ± 0.030	4.89 ± 0.99	11.9 ± 1.59
Ethanol (n=5)	0.194 ± 0.014	0.200 ± 0.029	4.55 ± 0.27	12.5 ± 0.10
Females				
Control (n=4)	0.191 ± 0.013	0.206 ± 0.032	4.44 ± 0.70	12.1 ± 1.80
Ethanol (n=4)	0.163 ± 0.017*	0.217 ± 0.018	4.26 ± 0.75	11.7 ± 1.29
Cerebellum				
Males				
Control (n=6)	0.223 ± 0.016	1.190 ± 0.126	3.57 ± 0.59	14.5 ± 1.95
Ethanol (n=5)	0.219 ± 0.009	1.079 ± 0.005	3.10 ± 0.68	15.8 ± 0.77
Females				
Control (n=4)	0.212 ± 0.011	1.125 ± 0.085	3.07 ± 0.27	14.3 ± 1.65
Ethanol (n=4)	0.169 ± 0.022*	0.935 ± 0.087*	2.44 ± 0.40*	12.6 ± 1.72

*$p<0.05$.

(Table 2, Fig. 1). When the female hindbrains were divided into cerebellum and brainstem, it was obvious that cerebellar growth was more severely impaired. The female brainstem samples did not differ in DNA, cholesterol or protein content, but the cerebellums were different in both DNA and cholesterol content, with the ethanol-treated group having the lowered amounts. Despite the lower amount of total cholesterol in the samples, the cholesterol/DNA ratio was not decreased in the 60-day-old ethanol-exposed females. Thus, although the total amount of cells in the cerebellum of ethanol-exposed females appears to be decreased, the degree of myelination and/or arborization per cell appears to be normal.

Evidence from other laboratories indicates that male brains do not always recover from perinatal exposure to ethanol. Both hippocampal and cerebellar cell populations in males exposed to ethanol perinatally and/or postnatally have been permanently altered [8, 9, 10, 14]. The ability of brains of ethanol-exposed males to catch up in this study is most likely due to the time of ethanol exposure rather than to other variables (such as the dose, differential sensitivity, etc.), since at 18 postnatal days males and females had similar brain deficits. One possible factor that could account for the sex difference is the hormonal influence that may regulate brain growth [7]. Another factor is the timing of the brain growth spurt itself, which could be different for male and female rats. Only further work can determine the role these variables play in the "catch-up" growth seen in the ethanol-exposed males. It should be noted that "catch-up" growth is not a unique phenomenon in the rat and has been shown with prenatal irradiation [17] and malnutrition [11,39].

Whether the change in open field behavior of the ethanol-exposed females is directly related to their impaired brain growth or to other unmeasured effects of the ethanol exposure (i.e., changed adrenal hormonal levels, etc.) cannot be stated based on the present data. However, since there were no differences in either the nose poke or passive avoidance tasks, the rather large brain deficits found have at best only subtle influences on gross behavior of rats. Further studies are needed to explore more complex behaviors and to determine if different postnatal exposure times in males will show similar effects as those found in the females of this study.

ACKNOWLEDGEMENTS

The authors wish to thank Kristin Lindberg and Challys Samson for their help in performing these studies.

REFERENCES

1. Abel, E. L. Prenatal effects of alcohol on open-field behavior, step-down latencies and "sleep time." *Behav Neural Biol* **25:** 406–410, 1979.
2. Abel, E. L. Fetal alcohol syndrome: Behavioral teratology. *Psychol Bull* **87:** 29–50, 1980.
3. Abel, E. L., editor. *Fetal Alcohol Syndrome*, vol. 2, *Animal Studies*. Boca Raton, FL: CRC Press, 1982.
4. Abel, E. L. and J. L. York. Absence of effect of prenatal ethanol on adult emotionality and ethanol consumption in rats. *J Stud Alcohol* **40:** 547–553, 1979.
5. Altman, J. Postnatal development of the cerebellar cortex in the rat I. The external germinal layer and the transitional molecular layer. *J Comp Neurol* **145:** 353–398, 1972.
6. Altman, J. and S. A. Bayer. Time of origin of neurons of the rat superior colliculus in relation to other components of the visual and visiomotor pathways. *Exp Brain Res* **42:** 424–434, 1981.
7. Anderson, R. A. Endocrine balance as a factor in the etiology of the fetal alcohol syndrome. *Neurobehav Toxicol Teratol* **3:** 89–104, 1981.
8. Barnes, D. E. and D. W. Walker. Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. *Dev Brain Res* **1:** 333–340, 1981.
9. Bauer-Moffett, C. and J. Altman. Ethanol-induced reductions in cerebellar growth of infant rats. *J Exp Neurol* **48:** 378–382, 1975.
10. Bauer-Moffett, C. and J. Altman. The effect of ethanol chronically administered to pre-weanling rats on cerebellar development: A morphological study. *Brain Res* **119:** 249–268, 1977.
11. Benton, S. W., H. W. Hoser, P. R. Dodge and S. Carr. Modification of the schedule of myelination in the rat by early nutritional deprivation. *Pediatrics* **38:** 801–807, 1966.
12. Bond, N. W. and E. L. Digusto. Prenatal alcohol consumption and open-field behavior in rats: Effects of age at time of testing. *Psychopharmacology* **52:** 311–312, 1977.
13. Bond, N. W. and E. L. Digusto. Effects of prenatal alcohol consumption on open-field behavior and alcohol preference in rats. *Psychopharmacology* **46:** 163–165, 1976.
14. Borges, S. and P. D. Lewis. A study of alcohol effects on the brain during gestation and lactation. *Teratology* **25:** 283–289, 1982.
15. Branchey, L. and A. J. Friedhoff. Biochemical and behavioral changes in rats exposed to ethanol in utero. *Ann NY Acad Sci* **263:** 328–330, 1976.
16. Clarren, S. K. and D. W. Smith. The Fetal Alcohol Syndrome. *N Engl J Med* **298:** 1063–1067, 1978.
17. D'Amato, C. J. Regeneration and recovery in the fetal nervous system after radiation injury. *Exp Neurol* **76:** 457–467, 1982.
18. Detering, N., R. M. Collins, R. L. Hawkins, P. T. Ozand and A. Karahasan. Comparative effects of ethanol and malnutrition on the development of cathecholamine neurons: A long-lasting effect in the hypothalamus. *J Neurochem* **36:** 2094–2096, 1981.
19. Diaz, J. and H. H. Samson. Impaired brain growth in neonatal rats exposed to ethanol. *Science* **208:** 751–753, 1980.
20. Dobbing, J. The later development of the brain and its vulnerability. In: *Scientific Foundations of Pediatrics*, edited by J. A. Davis and J. Dobbing. Philadelphia: Saunders, 1974, p. 565.
21. Driscoll, C. D., J. Chen and E. P. Riley. Passive avoidance performance exposed to alcohol during various periods of gestation. *Neurobehav Toxicol Teratol* **4:** 99–103, 1982.
22. Edwards, A. L. The analysis of variance for a randomized group design. In: *Experimental Design in Psychological Research*. New York: Holt, Rinehart and Winston, 1972, pp. 112–125.
23. Grant, K. A. and H. H. Samson. Ethanol and tertiary butanol induced microcephaly in the neonatal rat: Comparison of brain growth parameters. *Neurobehav Toxicol Teratol* **4:** 315–321, 1982.
24. Jones, K. L., D. W. Smith, C. N. Ulleland and A. P. Streissguth. Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* **1:** 1267–1271, 1973.
25. Lochery, E. A. and E. P. Riley. Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. *Neurobehav Toxicol* **2:** 107–115, 1980.
26. Lowry, O., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
27. Majewski, F. Alcohol embryopathy: Some facts and speculations about pathegenesis. *Neurobehav Toxicol Teratol* **3:** 129–144, 1981.
28. Randall, C. and W. J. Taylor. Prenatal ethanol exposure in mice: Teratogenic effects. *Teratology* **19:** 305–312, 1979.
29. Riley, E. P., N. R. Shapiro and E. A. Lochery. Nose-poking and head-dipping behaviors in rats prenatally exposed to alcohol. *Pharmacol Biochem Behav* **11:** 513–519, 1979.
30. Runyon, R. P. and A. Haber. Statistical inference with ordinally scaled variables. In: *Fundamentals of Behavioral Statistics*. Reading: Addision-Wesley, 1977, pp. 339–347.
31. Samson, H. H. and J. Diaz. Altered development of brain by neonatal ethanol ethanol exposure: Zn levels during and after exposure. *Alcoholism: Clin Exp Res* **5:** 563–569, 1981.
32. Samson, H. H. and J. Diaz. Effects of neonatal ethanol exposure on brain development in rodents. In: *Fetal Alcohol Syndrome*, vol. 3: *Animal Studies*, edited by E. L. Abel. Boca Raton; FL: CRC Press, 1981.
33. Samson, H. H., K. A. Grant, S. Coggan and V. M. Sachs. Ethanol induced microcephaly in the neonatal rat: Occurrence without withdrawal. *Neurobehav Toxicol Teratol* **4:** 115–116, 1982.
34. Searcy, R. L. and L. M. Berquist. A new color reaction for the quantitation of serum cholesterol. *Clin Chim Acta* **5:** 192–199, 1960.
35. Streissguth, A. P., S. Landesman-Dwyer, J. C. Martin, D. W. Smith. Teratogenic effects of alcohol in humans and animals. *Science* **209:** 353–361, 1980.
36. Sulik, K. K., M. C. Johnston and M. A. Webb. Fetal alcohol syndrome: Embryogenesis in a mouse model. *Science* **214:** 936–938, 1981.
37. Ulleland, C., R. P. Wennberg, R. P. Igo and N. J. Smith. The offspring of alcoholic mothers. *Pediatr Res* **4:** 474, 1970.
38. Ulleland, C. N. The offspring of alcoholic mothers. *Ann NY Acad Sci* **197:** 167–169, 1970.
39. Zamenhof, S. and D. Guthrie. Differential responses to prenatal malnutrition among neonatal rats. *Biol Neonate* **32:** 205–210, 1977.
40. Zamenhof, S. H., H. Bursztyn, K. Rich and P. J. Zamenhof. The determination of deoxyribonucleic acid and of cell number in brain. *J Neurochem* **11:** 505–509, 1964.