ETHANOL, A TERATOGEN: ITS EFFECT ON DEVELOPMENT IN RATS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

IN THE GRADUATE SCHOOL OF

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF

ARTS AND SCIENCE

BY

BINA K. MAYUR, M.SC.

DENTON, TEXAS

DECEMBER 1980

ACKNOWLEDGMENTS

I would like to express my sincere thanks as well as appreciation to Dr. Francine Lancaster, my thesis advisor and committee chairperson, for her advice and support in this endeavor.

Thanks are also in order for Drs. Walter Zetusky, Carrie Norris, both of TWU; Dr. Richard Wiggins of U.T.; and Dr. Thaddeus Samorajski of TRIMS for serving on my committee. Special thanks are due to Dr. Samorajski for funding this project; to Dr. Wiggins for the use of his well equipped laboratory and for his able guidance throughout the course of this project.

I am grateful for the help and suggestions received from Dr. Thomas Poder, Dr. Philip Patsalos, and Greg Fuller.

Finally, I would like to express my profound gratitude to the members of my family for their support and inspiration. In particular, I would like to thank my husband, Kiran, who suffered through this period performing numerous duties including cooking, cleaning, and providing transportation at odd hours in addition to putting up with my bad moods.

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Alcohol abuse and alcoholism are major health, social and economic problems facing the world today. At least 10-11 million people suffer from alcohol related problems in the U.S.A. with women accounting for 20-25% of the problem (Majchrowicz and Noble, 1979; Port, 1978). During the last two decades, there has been an estimated 83% increase in the proportion of problem drinkers who are women. The increase in alcohol related problems among women, the thalidomide tragedy of the 1960s and the effects of diethylstilbestrol on female offspring have underscored the fact that the fetus can be easily affected by drugs, and have renewed interest in the embryotoxicity of alcohol.

Purpose of the Study

The rising incidences in drug abuse by pregnant women initiated the present study. The purpose of the project was to investigate the effects of ethanol, a teratogen, on the development of rats. The effects of ethanol on the following parameters were studied:

(1) gestation period, (2) growth of offspring, (3) myelin synthesis and accumulation, and (4) weight of body organs.

Hypothesis and Model

The present study investigated the hypothesis that alcohol administered to pregnant rats would have an effect on the synthesis of myelin and growth of body organs in the offspring. To test the hypothesis, Long Evans female rats were used. Rats were chosen because of the large volume of ethanol data being accumulated on the species, and easy handling of the animal. The model of alcohol administration employed has been commonly used to study Fetal Alcohol Syndrome (FAS)(Chernoff, 1977; Druse and Hofteig, 1978; Randall, 1977). Myelin synthesis was examined in offspring born to female alcoholic rats using a double label isotope procedure (Wiggins et al., 1976; Wiggins and Fuller, 1979). Myelin accumulation was studied by the procedure of Norton and Podulso (1973a) and myelin proteins were measured by the methodology of Lowry et al. (1951).

Background

Fetal Alcohol Syndrome (FAS)

Chronic use of alcohol by pregnant females may cause Fetal Alcohol Syndrome (FAS) in their offspring. The fetus can be affected by alcohol because alcohol can cross placental barriers. FAS is associated with the following

signs: (1) prenatal growth retardation, postnatal growth retardation, or both, (2) central nervous system abnormalities, and (3) facial dysmorphology. If these characteristics are not found, the syndrome may be described as "possible fetal alcohol effects." FAS was clinically identified by Lemoine et al. (1968), and the term FAS was coined by Jones et al. (1973). In some cases the diagnosis of FAS can be made in the neonate, in others not until one or two years of age. Frequently, FAS babies have been identified without prior knowledge of maternal alcoholism.

Sufficient clinical evidence of FAS exists. Lemoine et al. (1968) identified 42 of 127 offspring with cleft palates, microopthalmia or limb malformations in children born to alcoholic mothers. The National Institute of Neurological Diseases and Strokes Perinatal Project studied 55,000 pregnancies (Niswander and Gordon, 1973). The study identified 23 typical FAS offspring. Additional FAS cases are being described by clinicians throughout the world.

Animal Models and FAS

Clinical evidence of the adverse outcome of alcohol abuse has given impetus to the study of effects of alcohol on the fetus at laboratory level. Tze and Lee (1975) reported smaller litter size in ethanol treated rats in comparison to those of pair-fed animals. The ethanol

offspring exhibited microcephaly and had a shrivelled appearance.

Kronick (1976) injected intraperitoneally a dosage of 0.03 ml/gm of body weight of 25% ethanol in pregnant mice on different gestation days, ranging from 7 to 12. Coloboma of the iris was most frequently observed in fetuses of the animals injected on days 8 and 9; absence of the forepaw was the main anomaly in offspring of mothers injected on day 10.

Chernoff (1977) conditioned mice to ethanol for 30 days prior to mating, using liquid diet. Pregnant dams were sacrificed on gestation day 18 and the uterine contents examined. Fetal resorptions increased with increasing ethanol concentrations. The pattern of growth deficiencies in the fetuses including the ocular, neural, cardiac and skeletal anomalies was similar to that observed in the Fetal Alcohol Syndrome in humans. Randall (1977) also administered ethanol in a liquid diet with adequate pair-fed controls. Fifteen of the 16 experimental litters had at least one malformed fetus; of the 29 control litters, only 5 had malformed fetuses. The fetuses of the ethanol group had limb anomalies which were absent in the controls. Detering et al. (1979) determined that when rats were fed a liquid diet containing 35% of calories as ethanol, the

offspring showed decreased brain weight and decreased body weight.

Diaz and Sampson (1980) fed ethanol to offspring obtained from nonalcoholic female rats on postnatal days 4 through 7 which resulted in 19% reduction in brain weight. There was no difference in body weights between the ethanol and control groups. Dexter et al. (1980) reported decreased litter sizes and birth weights in Sinclair (S-1) miniature swine dams consuming ethanol in a free choice situation. The stillbirths observed were 33% in the ethanol group as compared to 10% in the control groups.

These studies focused on the effects of ethanol on offspring viability, neonatal development and cellular growth, and indicated serious intellectual as well as physical impairment as a result of ethanol consumptions. However, the possibility that maternal malnutrition, stress or other environmental factors are associated with acute or chronic consumption of alcohol has not been evaluated. These studies also did not consider the effects of alcohol on the nervous system at the molecular level, although it is known that alcohol impairs the function of the brain.

Myelin Morphology and Function

The nervous system is divided into a central nervous system (CNS) and a peripheral nervous system (PNS). The

nervous system is made up of neurons and supporting cells such as oligondendroglia and Schwann cells. The neuron is composed of a cell body or perikaryon, from which extend a number of shorter processes known as dendrites, and a single, longer process known as an axon. The axon may be defined as that part of the neuron which conducts impulses. Myelin (Virchow, 1854) aids in the conduction of impulses. The segmental organization of myelin along axons aids in increasing the velocity of the impulse being transmitted between a nerve cell body and its target cell. Conduction in a myelinated fiber requires 1/300th of the energy required by a nonmyelinated fiber of the same size.

Myelin Structure and Composition

Myelin is a membranous structure, an extension of the plasma membrane. Myelin is produced by oligodendroglial cells in CNS while in PNS it is produced by Schwann cells. Besides forming myelin, the oligodendroglial cells and the Schwann cells have roles in: (1) repair and regeneration of the nervous system, (2) development of the nervous system, (3) uptake and release of neurotransmittors, (4) nutrition, and (5) phagocytosis (Varon and Somjen, 1979).

The composition of myelin has been extensively studied in rats (Norton and Podulso, 1973b; Nussbaum et al., 1963), bovines (Norton and Autilio, 1965), and man

(Fishman et al., 1975; O'Brien and Sampson, 1965a). These and other studies indicated that the composition of CNS myelin from various mammals is very similar, and consists of 15-30% proteins and 70-85% lipids. CNS myelin is characterized by its low amount of water (5-15%) and higher ratio of lipid to protein than found in any other subcellular fraction.

Myelination and Techniques of Study

Myelination occurs at specific times in various parts of the nervous system, beginning first in PNS (Flechsig, 1920). In rats, rabbits and mice, myelination begins 2 days postnatally in PNS, 10 postnatal days in CNS (brain) and in cerebral cortex at 15-21 days postnatal (Davison et al., 1966; Watson, 1903). In man, myelination occurs prenatally in the third trimester and continues until about 3 years of age (Flechsig, 1920).

The rate of synthesis of myelin components during development can be studied in vivo or in vitro. In vitro studies involve: (1) the use of slices from brain or spinal cord, (2)measurement of enzyme activities, and (3) the study of major protein or lipid fractions. The in vivo studies involve the use of radioactive precursors. The rate of incorporation of radioactive precursors is generally assumed to indicate the rate of synthesis at a

given time. Determination of the rate of synthesis using a single isotopic compound is largely dependent on such factors as efficiency of injection, isotope incorporation and metabolic variation in individual animals. However, these factors are internally controlled or totally eliminated in the double label procedure described by Wiggins et al. (1976).

In rats, the incorporation of radioactive precursors in myelin increases as myelination begins (8-10 days postnatally), continues rapidly during the period of active myelination (20-22 days postnatally) and then slows gradually as the rate of myelination decreases (28-30 days postnatally).

Myelination and Alcohol

Myelination is essential for the normal development of the body. The onset of myelination is considered an indication of functional maturation of the brain. The functional maturation can be impeded as the period of myelination is susceptible to metabolic insults during the brain growth spurt process (Dobbing, 1972). Alcohol acts at the cellular level by influencing metabolic pathways. Alcohol therefore may influence myelination. There are few reports on alcohol and myelination.

In 1957, Papara-Nicholson and Telford reported retardation of myelination along with lower birth weight in newborn guinea pig offspring whose mothers had been treated with ethanol. Rosman and Malone (1976, 1977) fed 21% of calories in the form of ethanol to pregnant rats. The offspring born to these rats had decreased myelination and lipid soluble brain proteins in comparison to the controls. Druse and Hofteig (1977) reported increased uptake of [^{3}H] leucine and [^{14}C] glucose in ethanol rat offspring of 18 and 25 days of age and decreased incorporation at 54 days in myelin subfractions. These offspring were born to females who were on ethanol (6.6% v/v) for one month prior to conception and throughout gestation. In 1978, the same researchers found near normal uptake of [^{3}H] and [^{14}C] in myelin subfractions of ethanol offspring of 18, 25 and 53 days of age when the females were fed 6.6% ethanol from day 5 of gestation to postnatal day 3.

Comparison of Nissl stained cortex of ethanol offspring to controls demonstrated reduced myelination in cortical white matter in ethanol groups. Myelination was completed in controls by 14 days while in ethanol offspring myelination did not appear until 23 days (Jacobson et al., 1979).

Sedmak et al. (1978) measured myelination by measuring the activity of the marker enzyme for myelin,

2', 3' cyclic nucleotide 3' phosphohydrolase. Myelination was decreased in mice fed ethanol for 123 days compared to pair-fed controls. However, the pair-fed animals had decreased total brain proteins unlike the ethanol group, indicating that the effect of ethanol was independent of nutrition and was different from malnutrition.

Myelination, Malnutrition and Alcohol

Myelination is vulnerable to malnutrition (Chase et al., 1967; Dobbing, 1963). Malnutrition is associated with alcoholism (Porta and Gomez-Dumm, 1968). Decreased intake of calories as a result of alcohol consumption may induce malnutrition. Insufficient nutrition during myelination results in a delay in myelination and a deficiency in the total quantity of myelin formed (Benton et al., 1966; Fishman et al., 1971; Wiggins et al., 1976). The effect of malnutrition is on synthesis rather than on degradation of myelin (Wiggins et al., 1976).

Myelination, Body Organs and Alcohol

Normal myelination is not only essential for the development of the nervous system, but it is also essential for the proper growth of body organs which are innervated by the peripheral nerves. Body organs are also vulnerable to drugs and malnourishment (Henderson et al., 1980; Wiggins

and Fuller, 1978). Studies on the effects of ethanol on developing organs with adequate controls are few.

The present study was designed to investigate the possible effects of ethanol on myelination and on body organs because of the lack of an adequate FAS model and the paucity of work on the effects of alcohol on developing brain and other body organs.

CHAPTER II

MATERIALS AND METHODS

Animals and Diet

Eighteen timed-pregnant, Long Evans rats (Charles River, Massachusetts) weighing approximately 190 gm were used in the experiments. Animals were housed individually in a temperature controlled room with light and dark cycles of 12 hours duration. The rats were fed with Lieber and Decarli's liquid diet (Bio Serve, New Jersey). The liquid diets were fortified with Dutch chocolate flavored Sego (Pet Incorporated, Missouri) to increase consumption. Ethanol (5.15% v/v of 190 proof) was added to the diet of experimental animals.

The control animals were fed the liquid diet in two patterns, pair-fed isocaloric and ad libitum. A lab chow ad libitum control was also used. The diet consumed by lab chow was not calculated. From day 18 of gestation, all rats were fed lab chow and water. Biweekly weight records were maintained for all animals. The offspring were toe clipped for individual identification. Litters were weaned on day 20. Experimental design, summary of treatment and definitions of groups are presented in Figure 2-1 and Table 2-1.

Figure 2-1. Protocol for the study of the effect of alcohol on myelination, organ and body weights.

PROTOCOL OF EXPERIMENTAL STUDY

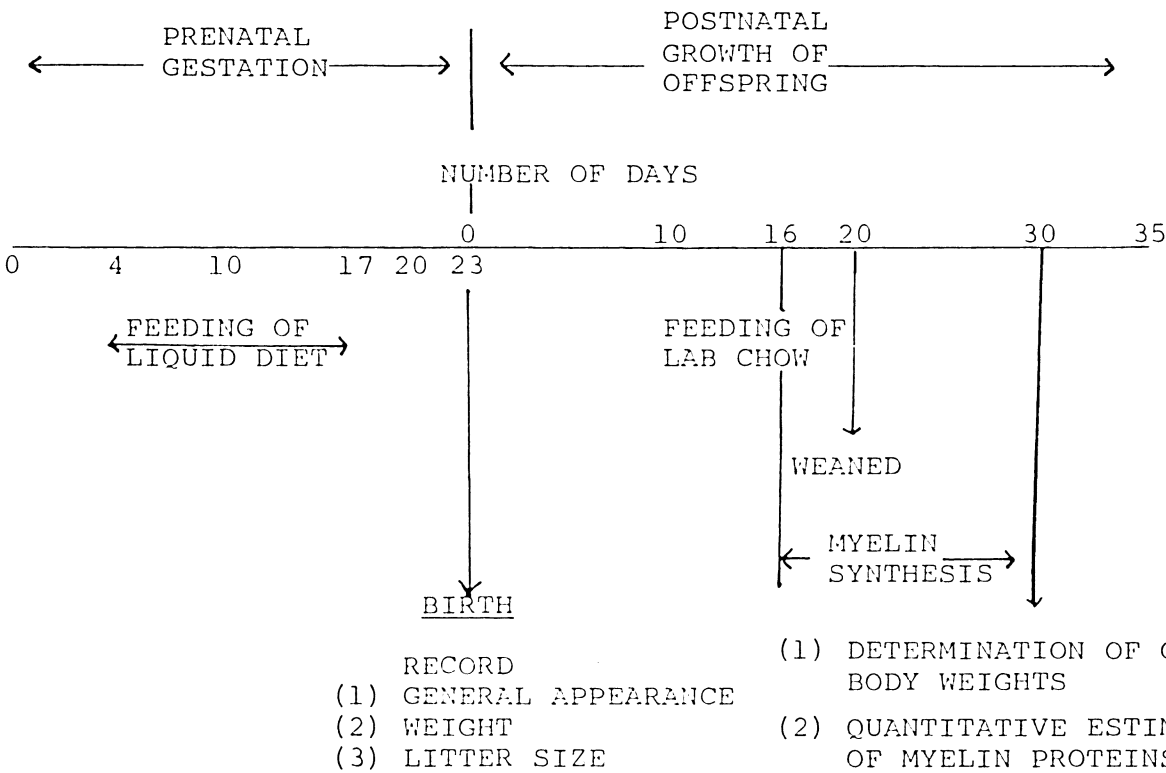


Table 2-1. Summary of treatment.

Groups (G)	Diet Fed[a]	Period of Treatment (Days)
GI = Experimental ethanol females (n = 6)	Liquid diet containing ethanol (5.15%; 190°proof)	14
	Lab chow and water (ad libitum)	27
GII = Control pair-fed females (n = 6)	Liquid diet containing isocaloric maltose-dextrin. Pair-fed with GI.	14
	Lab chow and water (ad libitum)	27
GIII = Control liquid diet ad libitum females (n = 3)	Liquid diet containing isocaloric maltose-dextrin. Fed ad libitum.	14
	Lab chow and water (ad libitum)	27
GIV = Control lab chow ad libitum females (n = 3)	Unlimited lab chow and water	41

[a]Liquid diet was fed from day 4 through day 17 of gestation. From day 18 of gestation until offspring were weaned, lab chow and water were fed. Lab chow ad libitum animals were fed unlimited lab chow and water from day 4 throughout the experiment.

Experiment 1: Myelin Protein Synthesis

Isotope Procedures and Radioactive Compounds

Each experimental offspring (alcoholic) was injected intraperitoneally with 1μCi/gm body weight of L-(1-^{14}C) leucine (57 Ci/mmole), and each control offspring (pair-fed and liquid diet ad lib) with 1μCi/gm body weight of L-[4, 5-^{3}H-(N)] leucine (135 Ci/mmole). Isotopes were purchased from New England Nuclear, Massachusetts.

The radioactive amino acids packaged in HCL were prepared for injection by drying the solutions under a stream of nitrogen at 40°C and alternately dissolving in water and redrying through two or three cycles. The final dried, acid free compounds were dissolved in 0.01 M phosphate buffered saline (pH 7.2).

Intraperitoneal injections of labelled leucine were made in volumes of 0.1 ml. Three hours after administration of radioactive leucine, the animals were sacrificed, the brains dissected and each brain cut into right and left halves and weighed. Each experimental half brain was combined with a half brain of pair-fed or a half brain of liquid diet ad lib. The combined brains were homogenized in 0.32 M sucrose (20% w/v) using a Teflon/glass homogenizer and subjected to subcellular fractionation.

Subcellular Fractionation

Synthesis of myelin, nuclear, synaptosomal, mitochondrial and microsomal fractions was examined using the double label isotope procedure (Wiggins et al., 1976; Wiggins and Fuller, 1979). The relative incorporation of [^{14}C] and [^{3}H] radioactivity into brain subcellular fractions was examined at a variety of postnatal ages, ranging from early to adult rates of myelin synthesis. Rat offspring at 16, 21 and 30 days of age were used for the study. At each age point, 3 rats were used. For control purposes, the reverse isotope study was conducted to determine any effects of differential utilization of the isotopes. For the reverse isotope study, rats of 22 days of age were used. In this case one alcoholic animal (GI) was injected intraperitoneally with 1μCi/gm body weight of [^{3}H] leucine and the controls (GII and GIII) with 1μCi/gm body weight of [^{14}C] leucine.

In the normal as well as the reverse isotope studies, the animals were sacrificed 3 hours after administration of leucine, the brains dissected, weighed and cut into left and right halves. The left side of an alcoholic rat brain (GI) was combined with left half brain of pair-fed (GII) rat and the right alcoholic brain part was combined with the right half brain of rat fed ad lib liquid diet (GIII). Each double brain sample was homogenized in 0.32 M sucrose

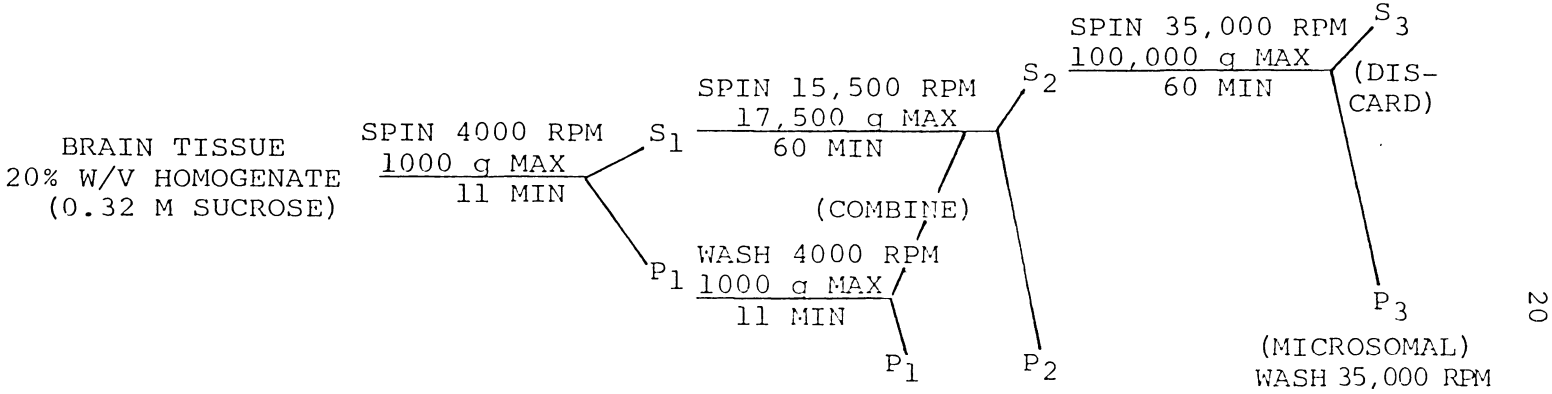
(20% w/v) using a Teflon/glass homogenizer, and subcellular fractions were prepared by ultracentrifugation on discontinuous sucrose gradient exactly as described previously (Wiggins et al., 1976; Wiggins and Fuller, 1979) including the osmotic shock and repurification on a second myelin gradient. Subcellular fractionation was carried out at 0-4°C. All particulate subcellular fractions (myelin, nuclear, synaptosomal and mitochondrial membranes) were washed by recentrifugation through three cycles in water and freeze dried (Figure 2-2).

Determination of Radioactivity

Radioactivity was determined from a portion of the sample following partial delipidation by extraction with 4 ml of ether-ethanol (3:2 v/v). Delipidated samples were dried, and a portion of the sample digested overnight at 40°C in 0.5 ml of 10% Protosol (New England Nuclear, Massachusetts). The counting cocktail was 10 ml of Omnifluor (New England Nuclear) scintillation cocktail in toulene. Radioactivity was measured with a Searle Mark III liquid scintillation counter. Data were automatically computed as [$^{14}C/^{3}H$] dpm ratios. The dpm ratios indicated the [^{14}C]radioactivity resulting from incorporation of leucine into proteins of the GI offspring and [^{3}H] radioactivity from incorporation by the GII and GIII offspring. The dpm

Figure 2-2. Procedure for subcellular fractionation of brain tissue. Diagram adapted from Wiggins et al. (1976).

SUBCELLULAR FRACTIONATION



COMBINE P_1A and P_2A AND:

1. WASH AT 25,000 RPM, 15 MIN, 75,000 g MAX
2. WATER SHOCK FOR 30 MIN
3. SPIN AT 10,500 RPM, 10 MIN, 12,000 g MAX
4. LAY OVER A MYELIN GRADIENT (0.85 M AND 0.32 M)

RESUSPEND (2 ML) — 120 MIN | SPIN 20,000 RPM 53,000 g MAX

0.32 M	P_1A (MYELIN)
0.8 M	P_1B (NUCLEAR)
1.2 M	P_1C (DISCARD)

RESUSPEND (2 ML) — 120 MIN | SPIN 20,000 RPM 53,000 g MAX

0.32 M	P_2A (MYELIN)
0.8 M	P_2B (SYNAPTOSOMAL)
1.2 M	P_2C (MITOCHONDRIAL)

5. SPIN AT 25,000 RPM, 30 MIN, 75,000 g MAX
6. WASH MYELIN 2 OR 3 TIMES EACH 15 MIN CYCLE
7. RECOVER OTHER FRACTIONS (NUCLEAR, SYNAPTOSOMAL AND MITOCHONDRIAL) AND REPEAT STEP 6 ONLY

ratios for each fraction were normalized with that of the corresponding microsomal value of the double brain homogenate. The result for each sample is a percentage value, which is a measure of relative synthesis for each subfraction of the experimental rat compared to the same fraction of the control.

Experiment II: Quantitative Estimation of Myelin Proteins

To measure myelin accumulation, offspring of 30 days of age were used. Six animals from each group, GI, GII, GIII, and GIV, were sacrificed; the brains were dissected, weighed and subjected to subcellular fractionation. Subcellular fractionation was carried out at 0-4°C. A 20% homogenate in 0.32 M sucrose (w/v) was made of each brain tissue using a Teflon/glass homogenizer. The homogenates were layered over 0.85 M sucrose and subcellular fractions prepared by ultracentrifugation on discontinuous sucrose gradients exactly as described previously (Norton and Poduslo, 1973a; Wiggins and Fuller, 1978). Myelin so obtained was freeze dried and partially delipidated by extraction with 4 ml ether-ethanol (3:2 v/v). Delipidated samples were dried and digested overnight at 10-15°C in 1 ml 1 N NaOH. Myelin protein was determined by the method of Lowry et al. (1951). Bovine serum albumin (Sigma) was used as a calibration standard in the determination.

Myelin content of brain tissue was reported as mg of myelin protein/gm of wet tissue.

Experiment III: Organ and Body Weights

Body organs including brain, pituitary, liver, spleen, heart, adrenals and kidneys were dissected from 30 day old rat offspring of the four groups. The various organs were weighed and compared by one way analysis of variance (ANOVA).

Statistical Analysis

Statistical comparisons of results among the four animal groups were determined by the t test for two means. A probability (p value) of <0.05 was used as the minimal level of significance (degees of freedom = $n_1 + n_2 - 2$).

CHAPTER III

RESULTS

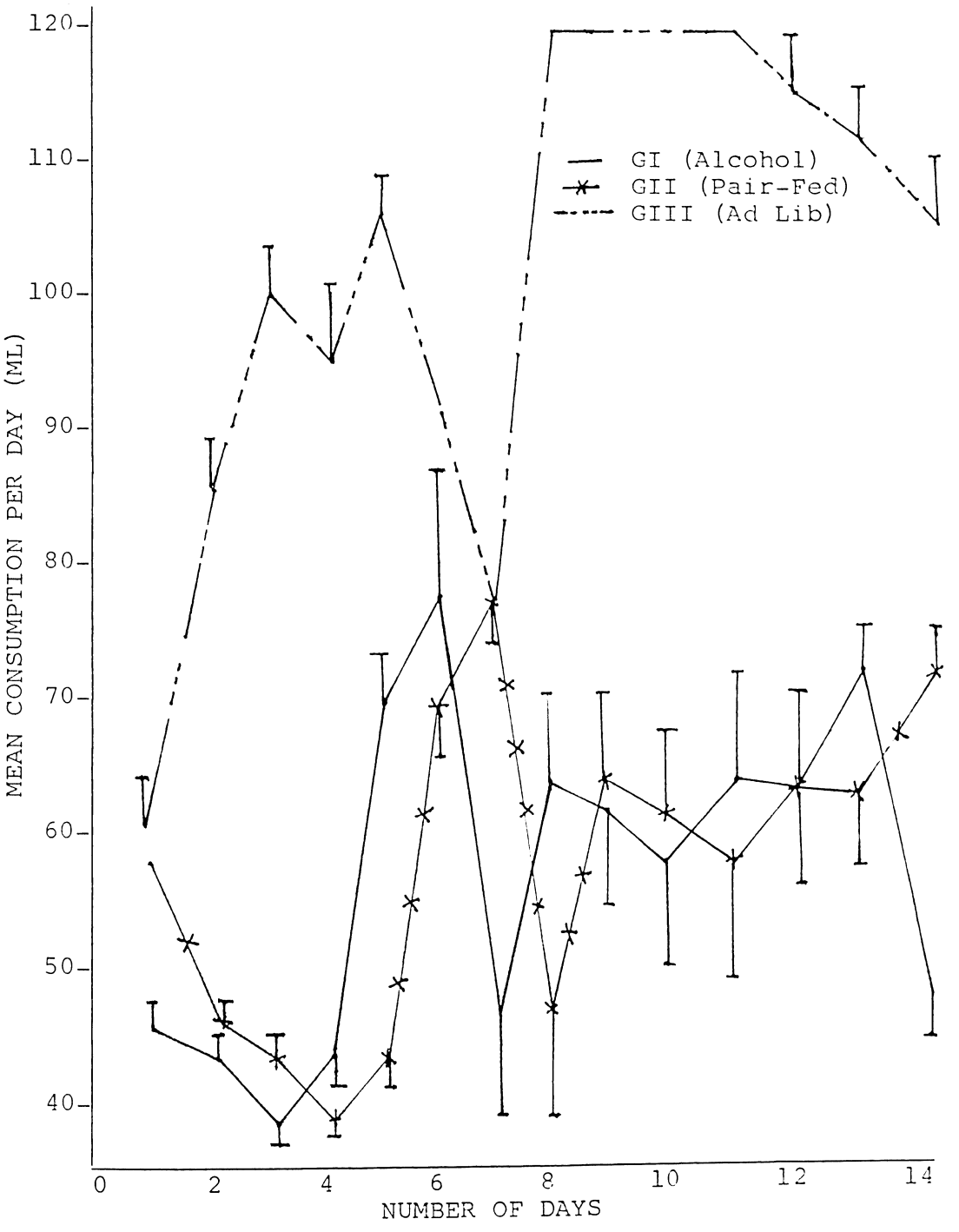
Results of experiments carried out to determine the effects of ethanol on the development of rats are presented in four parts: (1) data on growth, (2) myelin synthesis in rat offspring of 16, 21, 22 and 30 days of age, (3) myelin accumulation in rat offspring of 30 days of age, and (4) determination of organ and body weights of rat offspring at 30 days of age.

Data on Growth

Consumption of Liquid Diet

Consumption of liquid diet by pregnant rats (GI, alcoholic; GII, isocaloric; and GII, ad libitum on liquid diet) for a period of 14 days was as shown in Figure 3-1. During the first 4 days, the consumption of liquid diet by GI was lower than the intake measured on other days. In accordance with the experimental protocol, GII, the isocaloric control, was fed the same amount of diet that GI had consumed the previous day. GII consumed all the food that was allocated. The average daily consumption of liquid diet per group was: GI, 57 ±3 ml; GII, 58 ±3 ml; and GIII, 102 ±2 ml. Although

Figure 3-1. Consumption of liquid diet by pregnant rats in ml. GI (alcoholic), GII (pair-fed) and GIII (ad lib) dams consumed the diet for a period of 14 days starting from day 4 through day 17 of gestation.


120
110
100
90
80
70
60
50
40
MEAN CONSUMPTION PER DAY (ML)
GI (Alcohol)
GII (Pair-Fed)
GIII (Ad Lib)
0
2
4
6
8
10
12
14
NUMBER OF DAYS

GI and GIII were fed ad libitum, the diet consumed by GI per day was only 56% of the value in GIII.

Maternal Weight Gain

The predelivery weight gains in pregnant rats were 104 gm (54%) in GI, 104 gm (56%) in GII, 145 gm (70%) in GIII and 112 gm (56%) in GIV. The near identical weight gain by GI and GII indicated that the pair-fed isocaloric technique was successfully established. The post delivery gains in weight were: GI, 41 gm (16%); GII, 39 gm (16%); GIII, 12 gm (4%); and GIV, 31 gm (13%). Although the pair feeding was stopped on gestation day 18, the post delivery weight gains by GI and GII were comparable (Figure 3-2).

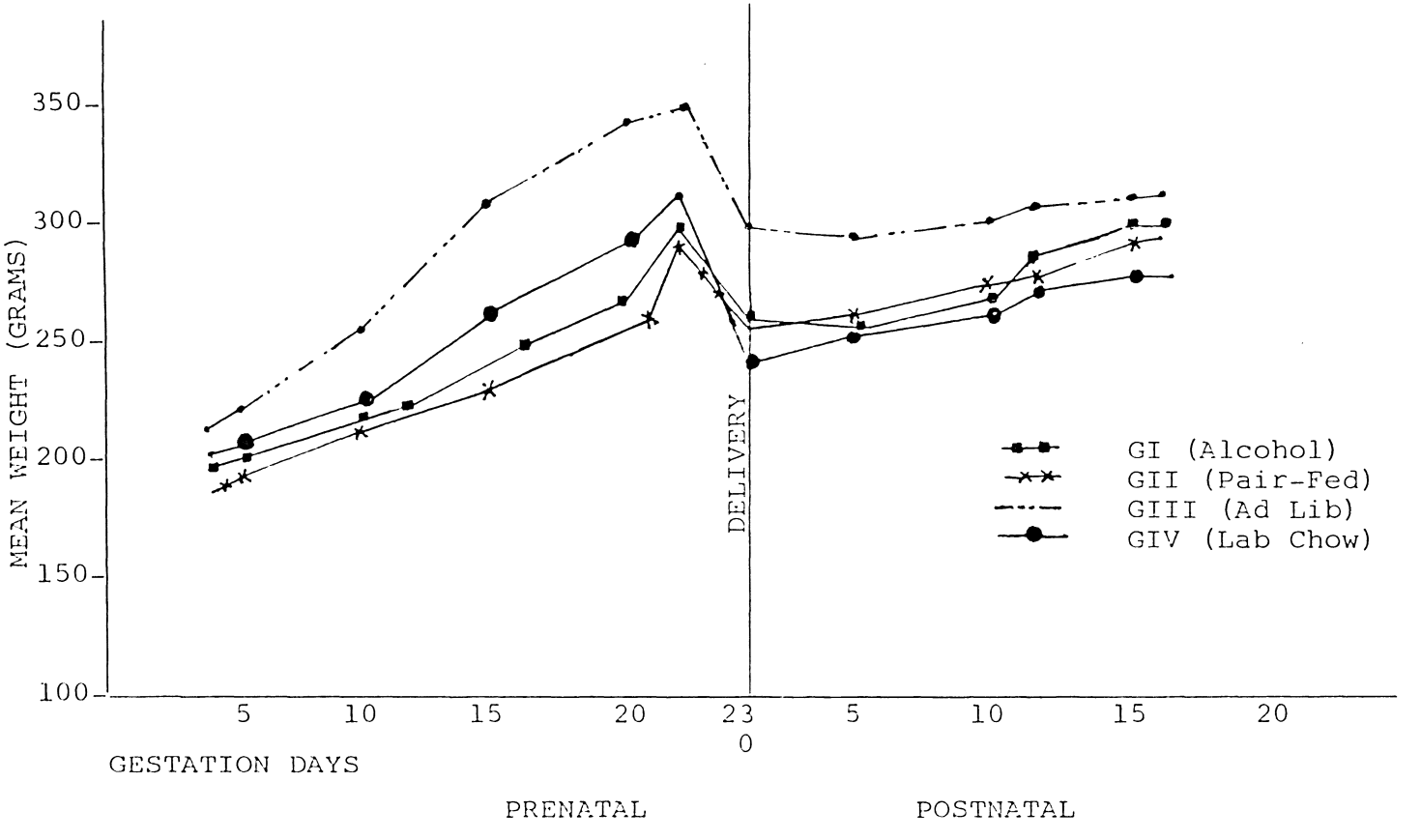
Gestation Period

GI, GII, GIII and GIV pregnant dams delivered during 22-24 gestation days.

Litter Size on Delivery Day

Of the dams, 50% of GI (three out of six) delivered with a mean litter size of 9.0 ±1.7; 50% of GII (three out of six) delivered with a mean litter size of 7.3 ±2.3; the remaining 50% of GI and GII dams did not deliver. Of the remaining dams, 100% of GIII (three out of three) and GIV (three out of three) delivered with mean litter sizes of 8.6 ±1.8 and 8.6 ±1.4, respectively. The litter sizes

Figure 3-2. Comparison of weight gain in female dams. GI, GII, GIII and GIV are alcoholic, pair-fed, liquid ad lib and lab chow dams, respectively. Standard error bars for each point encompass a range only slightly greater in size than the data symbols, and consequently these are omitted from the figure.


MEAN WEIGHT (GRAMS)
350
300
250
200
150
100
5
10
15
20
23
0
5
10
15
20
DELIVERY
GESTATION DAYS
PRENATAL
POSTNATAL
GI (Alcohol)
GII (Pair-Fed)
GIII (Ad Lib)
GIV (Lab Chow)

among all four groups were comparable in spite of the treatments (ethanol and isocaloric feedings).

Observation at Birth

No gross abnormalities were evident in any offspring. However, it was generally possible to distinguish ethanol offspring due to their smaller size. In GIII, one still-birth and two offspring with pale (whitish) skin were observed. One of the GI dams delivered offspring which were particularly low in weight. These offspring might have been affected by ethanol to a larger extent and hence, by postnatal day 3, all were dead. The mean weights of offspring per group (n = 3) on day 1 were as follows: GI, 5.5 ±0.5 gm; GII, 6.2 ±0.4 gm; GIII, 6.2 ±0.2 gm; and GIV, 6.8 ±0.3 gm. The differences were not statistically significant.

Offspring Survival Pattern

Survival of offspring depends on a number of factors. One of the factors is the concentration of ethanol used in the treatment of pregnant dams. As fetal loss was heavy in pilot studies in offspring exposed to ethanol and isocaloric treatments, the calories derived from ethanol were reduced from 35% to 27% for the present studies.

Total number of offspring per group on day 1 was as follows: GI, 37; GII, 22; GIII, 26; and GIV, 26. One of

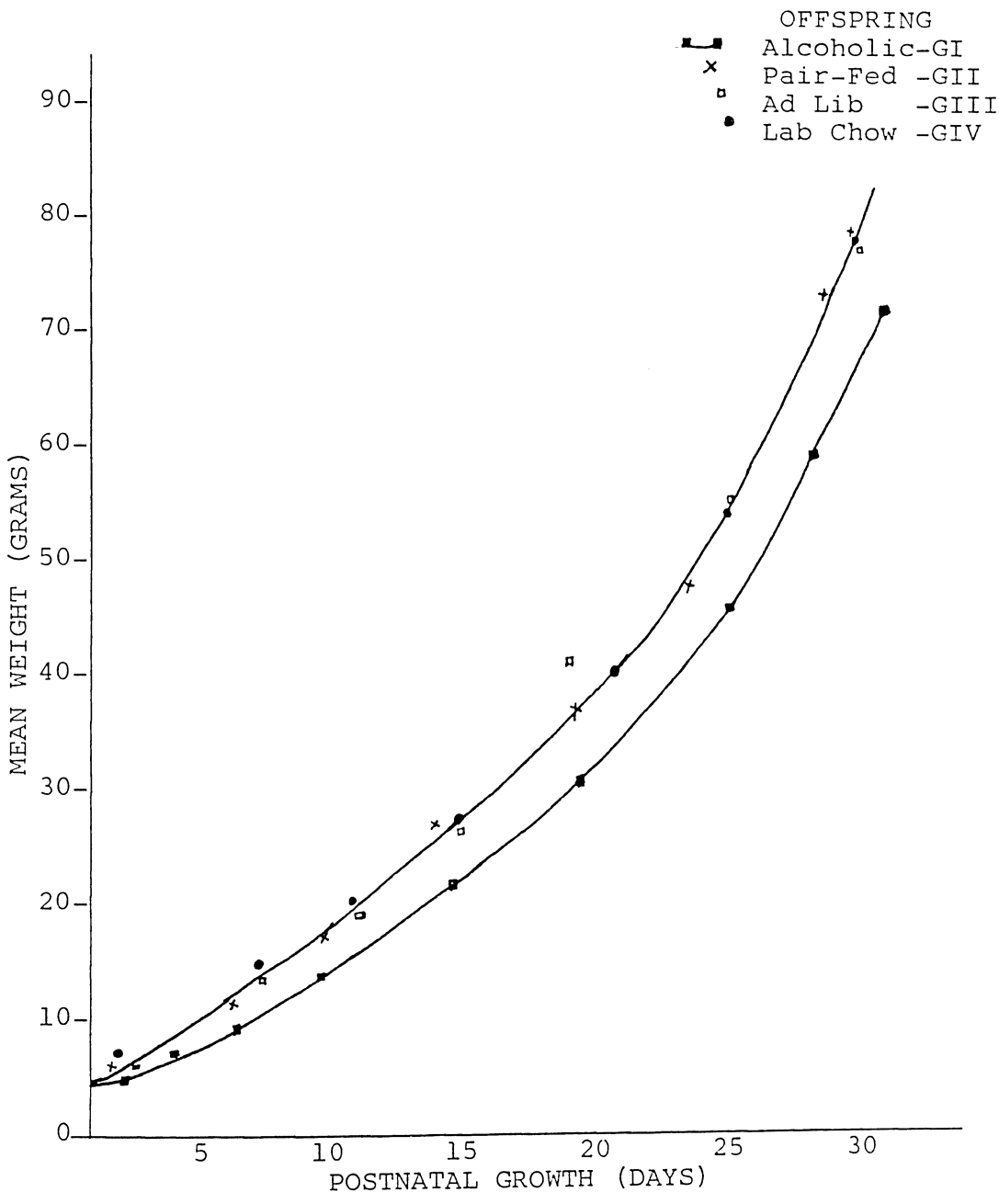
the females in GI lost eight of her offspring on day 2 and two more on day 3. Hence, the data on this animal were not used in statistical calculations. There were two more offspring deaths in GI by day 10. GIII had one stillbirth and two offspring with pale white skin which were dead by day 7. There were no postnatal deaths in GII and GIV. Past the tenth postnatal day there were no deaths among offspring of any group. In order to maintain a constant litter size, offspring were culled.

Weight of Offspring

Offspring growth of experimental (GI) and control (GII, GIII and GIV) groups were as shown in Figure 3-3. The offspring born to dams that were fed alcohol from day 4 through day 17 of gestation consistently weighed less than those of control groups, although the differences were not significant. GII dams, in spite of having been pair-fed to GI, had offspring which were comparable in weight throughout the study period to GIII and GIV.

One of the GI dams had offspring which weighed more than the offspring of the other two GI dams. As a result the pooled data of body weights of offspring of GI dams were not significantly different from those of GII and GIII. However, the body weights of GI offspring used in this study of myelin synthesis differed significantly from

Figure 3-3. Comparison of weight gain in experimental (GI) and control (GII, GIII and GIV) offspring. Standard error bars for each point encompass a range only slightly greater in size than the data symbols, and consequently these are omitted from the figure.


OFFSPRING
Alcoholic-GI
Pair-Fed -GII
Ad Lib -GIII
Lab Chow -GIV
MEAN WEIGHT (GRAMS)
0
10
20
30
40
50
60
70
80
90
5
10
15
20
25
30
POSTNATAL GROWTH (DAYS)

those of GII and GIII. These findings might have resulted from p values being calculated for fewer number of animals (n=3). Also, the effect of ethanol on individual offspring may have been different.

Eye Opening

By postnatal day 18, all the offspring had opened their eyes. Of the control animals, 85% had opened their eyes on day 15 and the remaining on day 16. GI offspring opened their eyes on days 16, 17 and 18, with a majority on day 17.

Results of Myelin Synthesis in Rat Offspring

Myelination, which begins postnatally in rats, was studied in animals of 16, 21, 22 and 30 days of age. At 16 days of age, the average brain weight of GI offspring (n=3) was 86% and 84% of that of GII and GIII, respectively (GI compared to GII, $p>0.05$; GII compared to GIII, $p>0.05$; GI compared to GIII, $p<0.050$). The average body weight of GI offspring was 69% and 73% of GII and GIII, respectively, and differed significantly from the two controls ($p<0.010$). Body weights, like brains weights, of the two control groups, GII and GIII, were comparable at day 16 indicating that the growth of GII offspring was not impaired, although GII mothers were pair-fed to GI (Table 3-1).

Table 3-1. Brain and body weights of offspring in the study of myelination.

Offspring Group	Postnatal Age (Days)							
	16[a]		21[a]		22[b]		30[a]	
	Body Wt (gm)	Brain Wt (gm)	Body Wt (gm)	Brain Wt (gm)	Body Wt (gm)	Brain Wt (gm)	Body Wt (gm)	Brain Wt (gm)
GI (alcoholic)	23 ±2	1.01 ±0.06	28.0 ±2	1.05 ±0.02	34	1.14	68 ±3	1.31 ±0.03
P values (GI/GII)	p<0.001	p=n.s.	p<0.005	p<0.01			p<0.005	p=n.s.
GII (pair-fed)	33 ±0.87	1.18 ±0.03	42.0 ±1	1.35 ±0.06	40	1.20	88 ±0.86	1.42 ±0.09
P values (GII/GIII)	p=n.s.	p=n.s.	p<0.025	p=n.s.			p=n.s.	p=n.s.
GIII (liquid ad lib)	32 ±0.26	1.20 ±0.01	48.0 ±1	1.34 ±0.06	49	1.31	90 ±2	1.35 ±0.02
P values (GI/GIII)	p<0.01	p<0.05	p<0.001	p<0.01			p<0.005	p=n.s.

[a]Each value is a mean of three determinations.

[b]Reverse isotope experiment, each value is a single determination. All weights are expressed as mean ± S.E.M. P values obtained by t test for two means, the weights in each case being the weights of the two groups indicated in brackets.

Table 3-2 shows the relative rates of synthesis of brain subcellular fractions of tests compared to control pairs. At day 16, the relative rate of myelin synthesis in GI offspring was about 68% of the rate in controls, suggesting that the rate of relative synthesis of myelin in GI offspring was depressed by about 32%, assuming that incorporation of radioactivity into microsomes was comparable in GI, GII and GIII offspring. The normalized values in GI offspring for other brain fractions--synaptosomal and mitochondrial--were close to 100%, indicating that the effect of ethanol is a specific effect on that membrane fraction alone. The nuclear fractions showed no definite trend in the utilization of radioactive leucine. As a result, the normalized values for nuclear fractions were inconsistant, making analysis difficult (Table 3-2).

In GII offspring of 16 days of age, the normalized relative rates of synthesis of myelin protein and other brain subfractions were close to 100% compared to GIII. The results reflected that isocaloric feeding had no influence on the synthesis of these fractions.

At 21 days of age, the average brain weight of GI offspring differed significantly and was about 78% of control values, GII and GIII ($p<0.010$). The average brain weights of GII and GIII offspring were comparable. The

Table 3-2. Relative rates of synthesis of brain subcellular membranes in offspring of rats exposed in utero to ethanol.

Subcellular Membrane Fractions	Group	Percentage ± S.E.M. Postnatal Age (Days) 16[a]	21[a]	22[b]	30[a]
Myelin Proteins	GI/GII	67 ±9	90 ±10	101	103 ±6
	GI/GIII	69 ±5	87 ±5	91	102 ±11
	GII/GIII[c]	107 ±15	97 ±6	90	98 ±5
Nuclear Proteins	GI/GII	75 ±0	101 ±19	129	92 ±6
	GI/GIII	109 ±0	86 ±9	65	81 ±8
	GII/GIII[c]	146 ±0	79 ±11	50	88 ±10
Synaptosomal Proteins	GI/GII	92 ±2	100 ±6	108	101 ±4
	GI/GIII	101 ±5	105 ±3	105	99 ±2
	GII/GIII[c]	110 ±7	106 ±7	97	98 ±5
Mitochondrial Proteins	GI/GII	92 ±6	102 ±6	110	98 ±2
	GI/GIII	98 ±6	104 ±0.3	106	101 ±5
	GII/GIII[c]	108 ±7	101 ±6	96	103 ±6

[a]Each value is a mean of three determinants.

[b]Reverse isotope experiment; each value is a single determination.

[c]Mathematical computation from GI/GII and GI/GIII. GI, GII and GIII are offspring born to alcoholic, pair-fed and liquid ad lib dams, respectively.

average body weight of GI offspring was 66% and 58% of GII and GIII, respectively (GI compared to GII, $p<0.005$; GI compared to GIII, $p<0.001$). Unlike the values of body weights at 16 and 30 days, the body weight of GII offspring

at 21 days of age differed significantly from GIII ($p<0.025$)(Table 3-1).

The relative rate of myelin synthesis in GI offspring of 21 days of age was about 88%, an increase of about 20% from 16 days of age (Table 3-2). The other subcellular fractions (synaptosomal, mitochondrial and microsomal) were close to 100%. These results suggested a "catch up" in myelin synthesis for ethanol offspring in spite of depressed myelin synthesis at day 16. The relative rates of synthesis of myelin proteins and other subcellular fractions were normal in control offspring of 21 days of age, suggesting that ethanol depressed myelin synthesis.

The reversed isotope study, a control study, was conducted in rat offspring of 22 days of age. The dpm ratios ($^{14}C/^{3}H$) in this case for GI/GII were: myelin, 1.805; nuclear, 1.408; synaptosomal, 1.694; mitochondrial, 1.666; and microsomal, 1.838. The dpm ratios ($^{14}C/^{3}H$) for GI/GIII were: myelin, 1.748; nuclear, 2.469; synaptosomal, 1.513; mitochondrial, 1.511; and microsomal, 1.488. The dpm ratios ($^{14}C/^{3}H$) for nonreversed study ranged from 1.0 to 2.13. The dpm ratios ($^{14}C/^{3}H$) of nonreversed study on days 16, 21 and 30 for myelin subcellular fractions were about 1.45, 1.75 and 1.3, respectively, while other subcellular fractions were about 1.9, 2.1 and 1.3, respectively.

The values of the two isotopic studies indicated that the dpm ratios of the reverse isotope study had reversed from a larger number to a smaller number. Hence, the dpm ratios obtained in these studies were not due to differences in the utilization of radioactive leucine, but were due to metabolic differences as a consequence of the treatment.

The average brain weight of GI offspring at 22 days of age was 95% of GII and 87% of GIII, while the average body weight was 84% of GII and 69% of GIII offspring. The period of 20 to 23 postnatal days is considered the period of maximum myelin accumulation rate in rats (Norton and Poduslo, 1973a, 1973b). By day 22, the relative rates of synthesis of myelin and other subcellular fractions calculated from reversed dpm ratios were close to 100% in GI, GII and GIII offspring. The data suggested that in spite of reduced myelin synthesis in GI offspring at 16 days of age, myelination was normal by day 22.

Thirty day old offspring are considered to be forming myelin at a rate comparable to that in adult rats. At 30 days of age, average brain weight of GI offspring was 92% and 97% of GII and GIII (Table 3-1). In comparison with GII and GIII offspring, the average body weight of GI was reduced by about 24%, showing significant differences when compared to both controls ($p<0.005$). The brain and

the body weights of GII and GIII offspring were comparable. The relative synthesis of myelin and other subcellular fractions were close to 100% in all offspring groups, suggesting that the "catch up" in myelin synthesis was completed (Table 3-2).

Results of Myelin Accumulation

Myelin content in offspring of 30 days of age are as shown in Table 3-3. The accumulation of myelin proteins by day 30 were comparable in all four groups. GI offspring used in this study also showed significant differences in brain and body weights with respect to controls.

Results of Organ and Body Weights

The mean body and organ weights of GI, GII, GIII and GIV offspring of 30 days of age are presented in Table 3-4. There were no significant differences in the organ and body weights of the four groups except in spleen of ethanol offspring (GI). The mean weight of spleen in GI offspring was 20% higher than that of the value in GII.

Table 3-3. Myelin content (mg myelin protein/gm wet wt of brain tissue ± S.E.M.) of 30 day old alcoholic and control rats.

Offspring Group	Myelin Proteins mg/gm Mean ± S.E.M.	Body Weight gms Mean ± S.E.M.	Brain Weight gms Mean ± S.E.M.
GI	3.5 ±0.2 (n=6)	72 ±2[a] (n=6)	1.39 ±0.02[b] (n=6)
GII	3.5 ±0.2 (n=6)	82 ±2 (n=6)	1.50 ±0.04 (n=6)
GIII	3.7 ±0.2 (n=5)	84 ±3 (n=6)	1.51 ±0.03 (n=6)
GIV	3.2 ±0.1[c] (n=6)	85 ±4 (n=6)	1.49 ±0.04 (n=6)

[a] $p<0.005$, GI compared to GII

[b] $p<0.05$; GI compared to GIV.

[c] $p<0.05$, GIV compared to any other group. GI, GII, GIII, and GIV are offspring born to alcoholic, pair-fed, liquid ad lib and ad lib lab chow dams, respectively.

Table 3-4. Comparison of organ and body weights of 30 day old alcoholic and control rats.

Weights	Groups			
	GI	GII	GIII	GIV
Body (gm)	77 ±6	78 ±6	81 ±6	86 ±8
Brain (gm)	1.44 ±0.07	1.49 ±0.06	1.49 ±0.07	1.38 ±0.31
Liver (gm)	3.19 ±0.82	3.29 ±0.31	3.45 ±0.18	3.86 ±0.50
Heart (mg)	373 ±23	336 ±50	346 ±30	376 ±42
Left Kidney (mg)	399 ±42	413 ±46	453 ±32	438 ±42
Right Kidney (mg)	422 ±33	434 ±45	463 ±27	457 ±35
Spleen (mg)	367 ±87[a]	294 ±47	333 ±49	361 ±62
Pituitary (mg)	3.1 ±0.5	3.1 ±0.9	3.7 ±0.5	3.9 ±0.5
Adrenals (mg)	15.5 ±2.5	17.2 ±2.1	16.4 ±2.1	17 ±2.0

[a] $p \leq 0.03$, ANOVA; weight expressed as mean ± S.E.M., each figure is a determination of n=12. GI, GII, GIII and GIV are offspring born to alcoholic, liquid ad lib and ad lib lab chow dams, respectively.

CHAPTER IV

DISCUSSION

Administration of ethanol, supplying 27% of the total calories to pregnant dams from day 4 through day 17 of gestation, reduced the intake of food to 56% of GIII (ad lib controls). Ethanol is known to depress the intake of food, thus leading to malnutrition. Reduced intake of food containing ehtanol has been reported (Abel, 1978). Since GII (pair-fed) was fed the same amount of liquid diet that the ethanol group had consumed, malnutrition occurred to a similar degree in GI and GII.

The near identical weight gain by GI and GII pregnant dams indicated that the pair-fed model was successfully established. The failure of GI pregnant dams to gain as much weight as GIII at the end of the ethanol treatment might have been due to ethanol's depressant effect on food intake (56% of GIII). These results corroborate previous reports of depressed weight gain by pregnant rats treated with ethanol (Kronick, 1976; Tze and Lee, 1975).

The normal delivery period for Long Evans rats is 22-25 gestation days. All dams delivered during this period, indicating that ethanol did not influence the

gestation or delivery period in the current study. Ethanol is known to influence the delivery period in humans and is used to retard premature labor onset. Normal delivery periods, in spite of ethanol treatment, have been previously reported (Abel, 1978; Cutler, 1979). These results are not compatible with those of Streissguth et al. (1980).

Litter sizes were comparable in ethanol treated pregnant dams and controls, suggesting that the drug might have had no influence on the implantation of ova. The results of this study are in agreement with those of Pilstrom and Kiessling (1967) and Cutler et al. (1979), although a decrease in litter size has also been noted (Tze and Lee, 1975).

One of the GI offspring group had comparable weight to controls; as a result, the pooled or combined offspring body weight data of GI group were not significantly different from controls (Figure 3-3). These results are in agreement with those of Rosman and Malone (1977) and Cutler (1979) and differ from those reported by Detering et al. (1979). However, weights of some of the offspring of GI (n=3)(Table 3-1) used for the study of myelination differed significantly from those of controls. A possible explanation for the differing data might have resulted from the

effect of ethanol not being uniform among the ethanol littermates, although ethanol treatment was the same to all GI pregnant dams. Average litter weight is considered to mask the actual weight differences between normal and malformed offspring of ethanol dams (Randall, 1977).

Though the pooled body weights of ethanol offspring did not differ significantly from those of controls, the ethanol offspring weighed less throughout the period of study (Figure 3-2). The constant lower weight might be accounted for by: (1) poor suckling and feeding habits of ethanol offspring--these habits have been clinically observed in human FAS babies; (2) impairment in the production of milk; and (3) presence of ethanol in the lactating females. Slower growth of ethanol offspring was also reported by Jacobson et al. (1979). The pooled body weights of GII offspring were comparable to those in GIII and GIV. These observations suggested that the effects of ethanol were different from those of malnourishment. Restricted amount of liquid diet fed to GII dams did not influence the body weights of GII offspring, possibly because of "catch up" growth by the GII dams after the treatment was stopped or the GII dams were not severely malnourished.

Survival of offspring subjected to the treatment of ethanol depends on a number of factors: the concentration

of ethanol, the length of treatment, the age and the species of offspring and the effect of ethanol on pregnant females. Pilstrom and Kiessling (1967) found all offspring dead by day 5 when exposed to 15% ethanol. Rosman and Malone (1977) reported 100% fetal loss in offspring born to females fed 35% of calories as ethanol and 60% fetal loss with 10% or 21.3% of calories as ethanol. In the initial pilot studies, ethanol was given prenatally as well as postnatally. Hence, in the current study ethanol was given prenatally only (Table 2-2). The reductions in the length of the treatment and the number of ethanol derived calories might have resulted in a better survival rate in offspring of GI (73% by day 3) and GII (100% by day 3). Pilot study three was abandoned because of the loss of offspring in GII. Fetal loss (GII) might have occurred due to malnourishment induced by feeding a restricted amount of diet.

The development of the eye is related to the growth of the central nervous system. There is very little information on the effects of alcohol on the development of the eye, eye opening and eye-lids. It was observed that the ethanol offspring opened their eyes one day later than the control groups. Eye opening tends to be delayed regardless of the administration method used to expose offspring to ethanol in laboratory animals (Streissguth et al., 1980).

Brain function is associated with myelination. Depressed myelin synthesis observed in ethanol offspring in the present study (Table 3-2) might account for the delay. Whether the delay occurred as a result of the effects of ethanol on the eyes, or the eyelids, or the nerve tracts remains to be investigated. Eye anomalies account for 80% of the anomalies associated with human clinical cases of FAS. Impairment in brain protein synthesis as confirmed in this study might explain some of the eye anomalies observed in FAS.

Data on the synthesis of various brain subcellular fractions (Table 3-2) clearly demonstrated that the synthesis of myelin proteins in rat offspring was selectively inhibited by ethanol in utero, while sparing other fractions. The data, therefore, suggested that not all structures in the developing brain were equally vulnerable to ethanol. The effects of ethanol were observed until 21 days and not beyond, on myelin synthesis. The results indicated that the direct, prenatal exposure to ethanol (via mother) and indirect postnatal exposure (via mother's milk) was reversible or temporary. The effects of ethanol on myelin synthesis were reversible or temporary as myelin synthesis in GI offspring was comparable with the two controls at postnatal day 22 (reversed isotope study) and

postnatal day 30 (nonreversed study). Normal recovery of total myelin proteins (3.2-3.7 mg/gm)(Table 3-3) in offspring of 30 days of age further supported that the effect of ethanol was temporary or had reversed.

The data obtained in the study corroborate with those of Rosman and Malone (1976, 1977) and Jacobson et al. (1979). These results, however, are not compatible with those of Hofteig and Druse (1977). They observed an increase in myelination in ethanol offspring when fed ethanol (35% calories via mother) one month prior to conception and throughout gestation. The same workers (1978) reported near normal myelination in rats which were fed ethanol from the fifth day of gestation to the third postnatal day. Their results can best be explained by the fact that the effect of ethanol depends on the length of treatment, the species of the animal and the concentration of ethanol administered.

The depressed myelin synthesis observed in ethanol offspring might have been a prenatal or a postnatal effect of alcohol or a combined effect. These conclusions cannot be deduced from the present experiment as cross fostering or feeding ethanol to normal or untreated offspring were not a feature of the model. Rosman and Malone (1977) found that when untreated offspring were fed ethanol (21%

calories) postnatally at weaning there was reduction in myelin proteins at 32 and 40 days, but by day 60, myelination was normal. They reported similar results for offspring born to ethanol fed females. These observations and the results of the present study indicated that the effect of ethanol (27% of calories via mothers) on myelin synthesis was prenatal as well as postnatal.

The developing brain has a sequential pattern for growth of cells. The increase in cell number is first seen in neurons, and then in glial cells. Therefore, the effect on myelination by drugs or any other factor can be cell specific. In rats, the prenatal period is marked by the growth of neurons while the postnatal period is characterized primarily by the growth of glial cells. Since the hypothesis was that the effect of ethanol was prenatal as well as postnatal, the depressed myelin synthesis was accounted for by: (1) a temporary reduction in number of oligodendroglia, (2) reduction in number of oligodendroglia leading to impairment in production of myelin, or (3) reduction of neurons resulting in decrease of axons to be myelinated; hence, a decrease in myelin synthesis. Therefore, the selective inhibition of myelin synthesis and sparing of mitochondrial, synaptosomal and microsomal synthesis indicated that the effect of ethanol was on oligodendroglia and neuronal cells.

Chronic use of alcohol promotes malnourishment (Porta and Gomez-Dunn, 1968). The effects of alcohol are considered similar to the effects of malnourishment or the effects of ethanol are due to malnourishment. Pair-fed controls partly rule out the possibility of malnutrition, as the intake of food is the same as in the ethanol group. Although pair-fed dams (GII) consumed 56% of the diet that the ad lib control (GIII) consumed, the GII offspring were comparable to GIII offspring at most of the age points studied with respect to brain and body weights and myelin synthesis (Tables 3-1, 3-2). Comparable results in GII and GIII offspring indicated that: (1) the pair-fed dams were not severely malnourished and hence GII offspring were not severely malnourished, and (2) malnourishment, if any, in GII offspring did not affect brain and body weights and myelin synthesis in these offspring.

The depressed myelin synthesis in GI offspring and not in GII offspring indicated that: (1) the adverse effects observed in this model were due to ethanol alone and not due to reduced intake of diet, (2) the depressed myelin synthesis was not secondary to nutritional deficiencies, and (3) the effects of ethanol were independent of nutritional adequacy.

The significant differences in body weights between experimental and control offspring groups (GI and GII), but

not to a large extent in brain weights, support the "brain sparing" phenomena observed under adverse conditions.

Growth of body organs is essential for development. The growth of organs can be impaired by drugs, stress and environment. At day 30, various organ and body weights of GI, GII, GIII and GIV offspring were compatible except for spleen of GI (Table 3-4). The increase in weight by 20% observed in spleen of ethanol offspring differs from that reported by Wiggins et al. (1978) in an undernutrition study wherein spleen was reduced by 75% at 20 days. The results indicate that the effects of ethanol and undernutrition are different. The increase in spleen weight was probably because of an increase in the functional capacity of spleen to withstand the effects of ethanol.

Summary and Conclusion

The data obtained in the present study showed that: (1) the animal model employed was adequate for the study of the effect of alcohol on pregnancy, (2) consumption of alcohol by pregnant dams resulted in offspring having a 32% decrease in the relative rate of synthesis of CNS myelin proteins, (2a) slower growth, and (2c) delay in the opening of the eyes. The findings demonstrated that: (1) alcohol in utero can impair the growth of offspring, (2) CNS can readily be affected by alcohol, and (3) alcohol

alone was responsible for the adverse outcome in offspring of treated mothers as no overt malnutrition was observed in offspring of pair-fed mothers.

Although the mechanisms for the adverse actions of alcohol on pregnancy are not fully known, the data obtained in the study indicated that consumption of alcohol during pregnancy is harmful to the offspring. The results observed in experimental animals cannot easily be generalized to humans, but do indicate the need for further studies in the field of maternal ingestion of alcohol.

CHAPTER V

REFERENCES

Abel, E. 1978. Effects of ethanol on pregnant rats and their offspring. Psychopharmacology. 57:5-11.

Benton, J. W., H. Moser, P. Dodge, and S. Carr. 1966. Modification of the schedule of myelination in the rat by early nutritional deprivation. Pediatrics. 38:801-807.

Chase, H. P., J. Dorsey, and G. McKhann. 1967. The effect of malnutrition on the synthesis of a myelin lipid. Pediatrics. 40:551-559.

Chernoff, G. 1977. The fetal alcohol syndrome in mice: An animal model. Teratology. 15:223-229.

Cutler, M., F. Ewart, and J. Makintosh. 1979. Growth and behavioral effects of ethyl alcohol on the offspring of mice; a comparison with its short-term actions. Psychopharmacology. 66:35-39.

Davison, A. N., M. Cuzner, N. Banik, and J. Oxberry. 1966. Myelinogenesis in the rat brain. Nature. 212:1373-1374.

Detering, N., W. D. Reed, P. Ozand, and A. Karahason. 1979. The effect of maternal ethanol consumption in the rat on the development of their offspring. J. Nutr. 109:999-1009.

Dexter, J. D., M. Tumbleson, and J. Decker. 1980. Fetal alcohol syndrome. Alcoholism. 4:146-151.

Diaz, J., and H. Somson. 1980. Impaired brain growth in neonatal rats exposed to ethanol. Science. 208: 751-753.

Dobbing, J. 1963. The influence of early nutrition on the development and myelination of the brain. Proc. R. Soc. (London) Ser. B. 159:503-509.

Dobbing, J. 1972. Vulnerable periods of brain development. In Lipids, Malnutrition and the Developing Brain, Ciba Symposium. Elsevier, New York: pp 9-20.

Druse, M. J., and J. H. Hofteig. 1977. 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.

Fishman, M. A., H. Agrawal, A. Alexander, J. Golterman, R. Martenson, and R. Mitchell. 1975. Biochemical maturation of human central nervous system myelin. J. Neurochem. 24:689-694.

Fishman, M. A., P. Madyastha, and A. L. Prensky. 1971. The effect of undernutrition on the development of myelin in the rat central nervous system. Lipids. 6:458-465.

Flechsig, P. 1920. Anatomie des menschlichen. Gehirns und Rückenmarks, Georg Thieme, Leipzig.

Henderson, G. I., A. Hoyumpa, C. McClain, and S. Schenker. 1980. The effects of chronic and acute alcohol administration on fetal development in the rat. Alcoholism. 3:99-106.

Hofteig, J. H., and M. J. Druse. 1978. Central nervous system myelination in rats exposed to ethanol in utero. Drug Alcohol Depend. 3:429-434.

Jacobson, S., J. Rich, and N. Tovsky. 1979. Delayed myelination and lamination in the cerebral cortex of the albino rat as a result of the fetal alcohol syndrome. Curr. Alcohol. 5:123-133.

Jones, K. L., W. D. Smith, and C. N. Ulleland. 1973. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet. 1:1267-1271.

Kronick, J. B. 1976. Teratogenic effects of ethyl alcohol administered to pregnant mice. Am. J. Obstet. Gynecol. 124(7):676-680.

Lemoine, P., H. Harousseau, and J. P. Borteyru. 1968. Les enfants de parents alcoholiques: Anomalies observees a propos de 127 cas. Arch. Fr. Pediatr. 25:830-832.

Lowry, O. H., N. Rosebrough, A. L. Farr, and R. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Majchrowicz, E., and E. Noble (eds.). 1979. In Biochemistry and Pharmacology of Ethanol. Plenum Press, New York, pp ix.

Niswander, K. R., and M. Gordon (eds.). The collaborative perinatal study of the national institute of neurological diseases and stroke: The women and their pregnancies. DHEW Publ. No. (NIH) '73-79. pp 500-524.

Norton, W. T., and L. A. Autilio. 1965. The chemical composition of bovine CNS myelin. Ann. NY. Acad. Sci. 122:77-85.

Norton, W. T., and S. E. Poduslo. 1973a. Myelination in rat brain: Method of myelin isolation. J. Neurochem. 21:749-757.

Norton, W. T., and S. E. Poduslo. 1973b. Myelination in rat brain: Changes in myelin composition during brain maturation. J. Neurochem. 21:759-793.

Nussbaum, J. L., R. Bietk, and P. Mandel. 1963. Phosphatide in myelin sheaths and repartition of sphingomyelin in the rat brain. Nature. 198:586-587.

O'Brien, J. S., and E. Sampson. 1965a. Lipid composition of the normal human brain: Gray matter, white matter and myelin. J. Lipid Res. 6:537.

Papara-Nicholson, D., and I. Telford. 1957. Effects of alcohol on reproduction and fetal development in the guinea pig. Anat. Rec. 127:438-439.

Pilstrom, L., and K. Kiessling. 1967. Effect of ethanol on the growth and on the liver and brain mitochondrial functions of the offspring of rats. Acta Pharmacol. Toxicol. 25:225-232.

Port, P. 1978. Women with alcohol problems, a text book. Master's Thesis, University of Texas School of Public Health, Houston, Texas, pp 14-56.

Porta, E. A., and C. Gomez-Dumm. 1968. A new experimental approach in the study of chronic alcoholism. I. Effects of high alcohol intake in rats fed a commercial laboratory diet. Lab. Invest. 18:352-364.

Randall, C., J. Taylor, and D. Walker. 1977. Ethanol-induced malformations in mice. Alcoholism. 1:219-224.

Rosman, N. P., and M. J. Malone. 1976. An experimental study of the fetal alcohol syndrome. Neurology. 26:365.

Rosman, N. P., and M. J. Malone. 1977. Reversal of delayed myelinogenesis in the fetal alcohol syndrome. Neurology. 27:369.

Sedmak, P. A., D. Sedmak, H. Fritz, and G. Peterson. 1978. Myelination in chronically-alcoholic mice. Experientia. 34:1059-1060.

Streissguth, A., S. Landesman-Dwyer, J. Martin, and D. Smith. 1980. Teratogenic effects of alcohol in humans and laboratory animals. Science. 209:353-361.

Tze, W., and M. Lee. 1975. Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. Nature. 257:479-480.

Varon, S., and G. Somjen. 1979. Neuron-glia interactions. Neurosci. Res. Program. Bull. 17:1-50.

Virchow, R. 1854. Ueber das ausgebreitete Vorkommen einer dem Nervenmark analogen substanz in den tierischen Geweben. Virchows Arch. Pathol. Anat. 6:562.

Watson, J. B. 1903. Animal education: An experimental study of the psychical development of the white rat correlated with the growth of its nervous system. Chicago Univ. Contrib. Phil. 4:90-111.

Wiggins, R. C., and G. N. Fuller. 1978. Early postnatal starvation causes lasting brain hypomyelination. J. Neurochem. 30:1231-1237.

Wiggins, R. C., and G. N. Fuller. 1979. Relative synthesis of myelin in different brain regions of postnatally undernourished rats. Brain Res. 162:103-112.

Wiggins, R. C., S. L. Miller, J. A. Benjamins, M. R. Krigman, and P. Morell. 1976. Myelin synthesis during postnatal deprivation and subsequent rehabilitation. Brain Res. 107:257-273.