EFFECT OF MATERNAL ALCOHOL CONSUMPTION UPON
PLASMA LEVELS OF FOLATE, ZINC, MAGNESIUM,
GLUCOSE, AND AMINO ACIDS IN RAT DAMS AND
THEIR FETUSES

by

SANDRA MAUREEN MARQUIS
B.Sc., The University of Victoria, 1979

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
Division of Human Nutrition
School of Home Economics

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
August 1982
© Sandra Maureen Marquis, 1982
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of HOME ECONOMICS

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date AUGUST 2, 1982
ABSTRACT

The current study was conducted to determine the effect of maternal alcohol consumption upon plasma amino acids, glucose, folic acid, zinc and magnesium levels in rat dams and their fetuses. Female Sprague-Dawley rats were divided into three dietary treatment groups. Group 1 (Alcohol) was given a stock diet plus 10% (v/v) ethanol in drinking water. After one week, the concentration of ethanol was increased to 20% (v/v). Group 2 (Pair-fed) was given a stock diet plus an amount of cornstarch isocalorically equivalent to the ethanol consumed by Group 1. Group 3 (Control) was given the stock diet ad libitum. Pair-fed and control rats received water ad libitum.

The three dietary groups were maintained on their respective regimens for 4 weeks, after which mating of each animal to male Sprague-Dawley rats was begun. The appearance of sperm in the vaginal washings was taken as day 1 of pregnancy. At that time, the alcohol group was changed to 30% (v/v) ethanol. Throughout the experiment daily food consumption by alcohol-treated and pair-fed animals was recorded. Weekly body weights of all animals were also recorded.

On day 21 of gestation fetuses and placentae were removed and weighed. Blood samples were collected by cardiac puncture from all dams and fetuses. Alcohol-treated fetuses were significantly lighter in body weight than pair-fed or control fetuses. Maternal weight gain prior to pregnancy was not correlated with fetal body weight, while maternal weight gain throughout pregnancy was positively correlated with fetal weight.

Maternal alcohol consumption had no significant effect upon maternal or fetal plasma levels of folic acid, zinc, and most amino acids. Alcohol
consumption also had no effect upon maternal plasma glucose and albumin concentrations. Fetal plasma lactic acid was not significantly affected by maternal alcohol consumption. However, plasma magnesium levels were significantly elevated in alcohol-treated dams. Also, plasma proline levels were significantly reduced and plasma alpha-amino-n-butyric acid levels elevated in alcohol-treated dams. Maternal plasma alpha-amino-n-butyric acid and proline levels correlated with fetal body weight.

In alcohol-treated fetuses, plasma glucose levels were significantly lower than those in pair-fed and control groups. Fetal plasma glucose levels were positively correlated with fetal body weight. Plasma aspartic acid levels were also significantly lower in alcohol-treated fetuses compared to the other two groups. Fetal plasma lysine and alpha-amino-n-butyric acid levels correlated positively with fetal body weight.

No significant differences were found in intestinal conjugase activity among the three maternal treatment groups, indicating that alcohol did not inhibit the hydrolysis of folate polyglutamates. Plasma osmolality was significantly elevated in alcohol-treated dams. However, the effect of moderate maternal dehydration upon fetal growth is unknown.

It is concluded that maternal alcohol consumption produces distinct changes in some maternal and fetal plasma nutrient concentrations (ie. glucose, zinc, magnesium and some amino acids). The significance of these results is unknown. However, fetal hypoglycemia, produced by chronic maternal alcohol consumption, may contribute to the growth retardation seen in the Fetal Alcohol Syndrome.
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>viii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>ix</td>
</tr>
<tr>
<td><strong>CHAPTER</strong></td>
<td></td>
</tr>
<tr>
<td><strong>I  INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>II  LITERATURE REVIEW</strong></td>
<td></td>
</tr>
<tr>
<td>A) Historical Perspective</td>
<td>3</td>
</tr>
<tr>
<td>B) Early Studies on the Teratogenic Effects of Alcohol in Humans</td>
<td>6</td>
</tr>
<tr>
<td>C) Early Studies on the Teratogenic Effects of Alcohol in Animals</td>
<td>7</td>
</tr>
<tr>
<td>D) Studies Examining the Mechanisms by which Alcohol Causes FAS</td>
<td>10</td>
</tr>
<tr>
<td>1) The Direct Effect of Ethanol</td>
<td>11</td>
</tr>
<tr>
<td>2) The Effect of Reduced Maternal Food Intake</td>
<td>11</td>
</tr>
<tr>
<td>3) The Effect of Ethanol upon Hormonal Function</td>
<td>11</td>
</tr>
<tr>
<td>4) The Effect of Ethanol upon Placental Transfer of Nutrients</td>
<td>12</td>
</tr>
<tr>
<td>5) The Effect of Ethanol upon Maternal Intestinal Nutrient Absorption</td>
<td>13</td>
</tr>
<tr>
<td>6) The Effect of Ethanol upon Maternal Urinary Loss of Nutrients</td>
<td>14</td>
</tr>
<tr>
<td>7) The Effect of Ethanol upon Blood and Tissue Levels of Nutrients</td>
<td>14</td>
</tr>
</tbody>
</table>
III EXPERIMENTAL DESIGN AND METHODOLOGY

A) Treatment of Animals ........................................... 19
B) Collection and Preparation of Animal Tissues .................. 20
C) Assay Procedures .................................................. 21
D) Statistical Analysis ............................................... 23

IV RESULTS

A) Caloric Intakes and Maternal Body Weights .................... 25
B) Litter Size, Fetal Body Weights and Placental Weights ........ 28
C) Nutrient Levels in Maternal Plasma ............................ 38
   1) Folic Acid ...................................................... 38
   2) Zinc ............................................................ 38
   3) Magnesium ..................................................... 38
   4) Glucose ......................................................... 38
   5) Albumin ........................................................ 38
D) Maternal Plasma Osmolality ...................................... 39
E) Maternal Intestinal Conjugase Activity ........................ 39
F) Maternal Plasma Amino Acids .................................... 42
G) Nutrient Levels in Fetal Plasma ................................. 49
   1) Folic Acid ...................................................... 49
<table>
<thead>
<tr>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
</tr>
<tr>
<td>49</td>
</tr>
<tr>
<td>49</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>52</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>59</td>
</tr>
<tr>
<td>61</td>
</tr>
<tr>
<td>75</td>
</tr>
<tr>
<td>77</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Daily Food and Alcohol Consumption Prior to Pregnancy</td>
<td>30</td>
</tr>
<tr>
<td>Table 2</td>
<td>Daily Caloric Intake Prior to Pregnancy</td>
<td>31</td>
</tr>
<tr>
<td>Table 3</td>
<td>Daily Food and Alcohol Consumption During Pregnancy</td>
<td>32</td>
</tr>
<tr>
<td>Table 4</td>
<td>Daily Caloric Intake During Pregnancy</td>
<td>33</td>
</tr>
<tr>
<td>Table 5</td>
<td>Weekly Maternal Body Weights Before and During Pregnancy</td>
<td>34</td>
</tr>
<tr>
<td>Table 6</td>
<td>Fetal Body Weight, Litter Size and Placental Weight</td>
<td>37</td>
</tr>
<tr>
<td>Table 7</td>
<td>Maternal Plasma Folate, Zinc, Magnesium, Glucose and Albumin Levels on Day 21 of Gestation</td>
<td>40</td>
</tr>
<tr>
<td>Table 8</td>
<td>Maternal Plasma Osmolality on Day 21 of Gestation</td>
<td>41</td>
</tr>
<tr>
<td>Table 9</td>
<td>Maternal Intestinal Conjugase Activity on Day 21 of Gestation</td>
<td>41</td>
</tr>
<tr>
<td>Table 10</td>
<td>Maternal Plasma Amino Acid and Urea Levels on Day 21 of Gestation</td>
<td>44</td>
</tr>
<tr>
<td>Table 11</td>
<td>Fetal Plasma Folate, Zinc, Magnesium, Glucose and Lactic Acid Levels on Day 21 of Gestation</td>
<td>51</td>
</tr>
<tr>
<td>Table 12</td>
<td>Fetal Plasma Amino Acid and Urea Levels on Day 21 of Gestation</td>
<td>54</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1 Maternal Weight Gain Prior to Pregnancy .......................................................... 35
Figure 2 Maternal Weight Gain Throughout Pregnancy ...................................................... 36
Figure 3 Maternal Plasma Amino Acid Levels on Day 21 of Gestation ............................... 46
Figure 4 Fetal Plasma Amino Acid Levels on Day 21 of Gestation ..................................... 56
ACKNOWLEDGEMENTS

I wish to express my appreciation to my advisor Dr. Joseph Leichter, for his direction and advice throughout this project. I wish also to thank Dr. Patricia Gallo and Dr. Krishnamurti for serving on my advisory committee, and Dr. Melvin Lee for his valuable advise and assistance in collecting blood samples. Thanks are also due to Mrs. Virginia Green of Arts Computing for support in computer programming and statistical analysis. Thanks are also expressed to Mrs. Helen Smith for her assistance in the laboratory.

Lastly, I would like to thank my husband Paul for helping me with the computer, and for all his patience and support.

Financial support is acknowledged from the British Columbia Youth Employment Program.
INTRODUCTION

A relationship between maternal alcohol consumption and neonatal morbidity and mortality has been observed since ancient times (1,2). However, only in the past decade has it been established that maternal alcohol consumption can produce a distinct pattern of abnormalities in newborns. This pattern was first described in detail by Jones and Smith (5,6); they termed it the Fetal Alcohol Syndrome (FAS). Characteristics reported in FAS children included pre- and postnatal growth retardation, craniofacial anomalies (short palpebral fissures, midfacial hypoplasia), microcephaly, mental deficiency, joint abnormalities and cardiac defects (9).

Following its initial description, growth deficiency in children as a result of maternal alcohol consumption was confirmed by many researchers (4-6,8-14). However, animal studies became necessary to differentiate between the effects of alcohol and other risk factors and to determine the mechanism by which alcohol causes FAS. Studies using rats have reported pre- and postnatal growth retardation in the offspring of dams given ethanol (15-19,21-28). Increased rate of fetal resorption (15,16,19,24,26), and reduced litter size (16,20,24) have also been reported in rats as a result of maternal alcohol consumption. However, it remains unclear whether the retardation in growth and development found in FAS is due to a direct effect of ethanol or one of its metabolites, or whether it is a consequence of maternal malnutrition.

Animal studies employing a pair-fed control group have demonstrated that fetal growth retardation is not simply a result of underfeeding of the dams (16-18,21-23). However, maternal alcohol consumption might
cause retarded fetal growth by interfering with maternal or fetal nutrient utilization. Ethanol may reduce intestinal nutrient absorption, placental transfer of nutrients, or tissue nutrient uptake.

The present study was designed to determine the effect of maternal alcohol consumption upon maternal and fetal plasma nutrient levels in the rat model. Rats were given ethanol prior to and throughout gestation and compared to pair-fed and \textit{ad libitum} fed control animals. On day 21 of gestation maternal and fetal blood samples were collected and levels of folic acid, zinc, magnesium, glucose and free amino acids determined in plasma.

The nutrients examined were chosen on the basis of both animal and human studies demonstrating an association between alcohol consumption and poor nutrient status. In the nonpregnant animal, alcohol has been shown to decrease intestinal absorption of folic acid (39), glucose (40), zinc (36) and amino acids (37). Alcohol increases urinary loss of zinc (36,38,41,42) and of magnesium (43) in humans. In animals, alcohol inhibits hepatic gluconeogenesis (47,48), produces changes in plasma amino acid patterns (50-52), and reduces tissue uptake of amino acids (53-58). Thus, in the present study, correlations between maternal alcohol consumption, plasma nutrient levels, and fetal growth retardation were examined, in the hopes of providing an explanation for alcohol-induced fetal growth retardation.
LITERATURE REVIEW

Historical Perspective

A relationship between maternal alcohol consumption and neonatal morbidity and mortality has been observed since antiquity. Burton's Anatomy of Melancholy, published in 1621, cited Aristotle's observation that "foolish and drunken harebrained women most often bring forth children like unto themselves, morose and languid" (1). However, through every era attitudes towards alcohol and its effects have been debated and revised.

From 1720 to 1750 England was swept by a "gin epidemic". It was during this period that the first observations of a fetal alcohol syndrome appeared in the English-language literature. In England in 1751, Morris attributed a drop in birth rate and increase in the mortality of children under 5 years of age to the "enormous use of spiritous liquors" (2).

In the United States literature on alcohol and its action during pregnancy did not develop until somewhat later than it had in England. However, as early as 1787 a tract entitled "An Enquiry into the Effects of Spiritous Liquors upon the Human Body", advised against the consumption of alcohol by pregnant women. (2).

By 1842 in the U.S., evidence of damage caused by maternal drinking was being used to support the drive for total abstinence. In 1848, Howe published the first report of epidemiological research on the topic of parental drinking. He examined the family histories of 145 institutionalized mental defectives and found that 33 had "intemperaté" parents (2). From 1870 to 1920, journals were flooded with articles and letters
about alcohol. Journals, such as The Journal of Inebriety and The Scientific Temperance Journal, contained reports of parental drinking resulting in epileptic, insane, imbecilic and alcoholic children.

To buttress these assertions, a vast body of survey research accumulated. Long surveyed 200 doctors in 1876 (2). These doctors attributed 20 to 30% of inherited mental deficiency to parental alcoholism. In 1899, Sullivan (1) studied 21 alcoholic mothers and compared the mortality rates of their 125 children to those of the offspring of their non-drinking female relatives. He found that the death rate of the offspring of the alcoholic mothers was nearly 2.5 times higher. He did emphasize however, that the high mortality rate was not solely due to a toxic effect of alcohol, but was also related to a high incidence of child abuse. This, and other studies, raised the question of whether high infant mortality in alcoholic families was directly related to alcohol, or due to poor maternal care.

In 1903, Hodge reported the first animal experiments on the effects of alcohol upon offspring. He treated cocker spaniels with alcohol and reported that the offspring of alcohol-treated had less vigour and vitality than normal (2). Studies using rodents began with Nice in 1912 and continued until 1924 (2). However, the experimental design of these investigations varied greatly and results remained inconclusive.

In 1920 Prohibition went into effect in the United States. In both the U.S. and Britain, a dramatic drop in medical writing on the effects of alcohol upon offspring ensued, and interest shifted towards the effects of environmental factors upon development of the newborn (2). Some animal experimentation with alcohol continued, but it was discounted as crude and uncontrolled.
In 1942, Haggard and Jellinek (2) stated that no evidence had thus far been given which showed conclusively that maternal alcohol intake produced any abnormality in the child. They attributed damage to offspring to the poor nutritional status and environment of the alcoholic mother.

In the 1940's and 1950's toxic effects of alcohol on offspring were generally discounted. Then in the 1960's the rediscovery of the fetal alcohol syndrome began. In 1962, Schaefer (3) reported a case of a Yukon Indian showing signs of alcohol withdrawal shortly following birth. In 1970, Ulleland et al. (4) retrospectively identified 11 women alcoholics and found that 10 of their 12 children were small for gestational age.

In 1973, Jones et al. (5) coined the term fetal alcohol syndrome (FAS). In two papers Jones et al. (5) and Jones and Smith (6) described a pattern of malformations found in 16 children. The children were of varied ethnic origin. The mothers were chronic alcoholics (see Criteria for the Diagnosis of Alcoholism by the Criteria Committee, National Council on Alcoholism (7)), who had continued to drink heavily throughout their pregnancies. The characteristics of FAS described in these two papers were pre- and postnatal growth deficiency, microcephaly, craniofacial abnormalities (short palpebral fissures, maxillary hypoplasia), and joint and cardiac abnormalities. Jones et al. (5) noted that birth length was more severely retarded than body weight. Furthermore, the average linear growth rate and weight gain of children followed for one year remained at 65% and 38% of normal respectively.

These findings completed an historical cycle in which the effects of alcohol upon offspring regained scientific attention. If repetition of such a cycle is to avoided, experiments must be conducted to delineate
ethanol's mode of action and to differentiate the effects of ethanol from environmental and social variables.

Early Studies on the Teratogenic Effects of Alcohol in Humans

A neglected 1968 report of Lemoine et al. (2) was found to independently confirm the findings of Jones et al. (5). Lemoine et al. described characteristics of FAS in 125 French children. These findings and those of Jones et al. stimulated a series of retrospective studies confirming the existence of FAS. Jones et al. (8) reported on an additional 23 alcoholic women. Thirty-two percent of the children born to these women had features of FAS. In a review of cases to date, Hanson et al. (9) described FAS characteristics in a total of 41 children. In 1978, Erb and Andresen (10) estimated that 1/3 to 1/2 of the children born to alcoholic mothers were affected to some degree with FAS. However, Streissguth et al. (11) emphasized that there is a great range in the number and severity of symptoms seen in children with FAS, and that no pattern of alcohol consumption nor lower limit of amount of alcohol required to produce FAS had as yet been established.

Retrospective studies established the probable relationship between alcohol consumption and FAS characteristics, but prospective studies were needed to confirm the relationship. Oulette and Rosett (12) surveyed 200 women attending prenatal clinics and divided them into three groups, based upon their alcohol consumption (abstinent, moderate, or heavy). The 82 babies born to women of the three groups were subsequently studied at birth. A significantly greater incidence of reduced birth weight, length and head circumference was found in infants of heavy drinkers. However, Oulette and Rosett concluded that because of the possible
interrelationships among alcoholism, cigarette-smoking, malnutrition and socio-economic status in their study groups, a conclusive relationship between alcoholism and FAS was not determined.

Little (13) studied the relationship between the alcohol intake of 263 women before and during pregnancy and the birth weight of their offspring. Using multiple linear regression, the study controlled for the influence of maternal age and height, parity, and cigarette-smoking and the newborns' sex and gestational age. Little (13) found that an average maternal intake of one ounce of absolute ethanol/day during the three month period prior to pregnancy was associated with a 90.8 gram decrease in birth weight. The same amount of ethanol consumed during late pregnancy produced an average birth weight decrease of 160 grams. The fact that Little found alcohol intake during early pregnancy had no effect on birth weight, may have been due to the small sample of women who drank during this period. Little points out that birth weight per se is not strictly equivalent to infant health. However, the reduction in birth weight may represent minimal damage within a larger spectrum of growth retardation and fetal injury.

In a retrospective study Little et al. (14) found that the mean birth weight of the offspring of alcoholics who had abstained throughout pregnancy was 258 grams less than that of controls, while birth weight of children of non-abstaining alcoholics was 493 grams less than that of controls. Again these birth weights were adjusted for variables such as maternal age, weight, smoking habits, parity and the infant's sex.

Early Studies on the Teratogenic Effects of Alcohol in Animals

Studies to determine the mechanism by which alcohol causes FAS have now turned primarily to the use of animals. Animal studies allow
manipulation not ethically possible in human studies. Animal studies also have an advantage in that they allow the study of the effects of a single factor (maternal alcohol consumption) upon pregnancy and fetal growth. Human studies are often compounded by variables such as malnutrition, cigarette-smoking and socio-economic status.

Animal studies established that a syndrome comparable to the human FAS could be induced in rodent fetuses. In 1975 Chernoff (15) fed mice alcohol, with levels ranging from 15-35% of their total calories. He found that different mouse strains varied in their sensitivity to alcohol, but that alcohol could cause decreased fetal weight, increased fetal resorption, deficient ossification and neural and cardiac abnormalities. During this same time, Tze and Lee (16) documented the effects of maternal alcohol consumption upon rat fetuses. Rats were given 30 grams ethanol/100 ml. water as their only fluid source. The ethanol group was compared to isocalorically pair-fed and ad libitum fed control groups. The rats were mated 5 weeks after initiation of the dietary regimens and maintained on the regimens throughout pregnancy. Only 50% of mated alcohol-fed dams delivered, while 91% of pair-fed and 88% of mated control animals delivered. The ethanol-treated dams had significantly reduced litter size.

Some features of the fetal alcohol syndrome have been reproduced in animal models, but the results are contradictory because of the wide variety of experimental conditions employed (15-28). In particular, experiments have differed in the mode and time of alcohol administration, and the amount of alcohol consumed.

Rider's (24) findings may explain some of the variability reported in the literature on the effects of maternal alcohol consumption upon rodent fetuses. Rider reported that rats can adapt significantly to
alcohol feeding. She compared the reproductive performance of female rats started on 11% ethanol (as their sole fluid source) 13 weeks before mating, and rats given ethanol only from day 1 of gestation. The two groups of pregnant rats did not differ in weight gain throughout gestation. However, the dams started on 11% ethanol on day 1 of gestation had a significantly reduced number of completed pregnancies as well as smaller litter size and weight, and a lower pup survival rate, when compared to both *ad libitum* fed controls and to dams started on the ethanol thirteen weeks earlier.

In addition to the timing of ethanol administration, studies have differed in the mode of administration. Several investigators have administered ethanol by intubation. Abel and Greizerstein (21) intubated rats with 6 grams ethanol (30%)/kg. body weight daily beginning on day 5 of pregnancy. In comparing the alcohol group to a pair-fed group, they found no significant differences in maternal weight gain, placental weight or litter size, but they did find that the fetuses of intubated dams had significantly lower body weights and lengths. Ethanol intubation allows very accurate monitoring of ethanol intake. However, the application of such a high concentration of ethanol directly to the gastrointestinal tract may cause some histological damage which would not otherwise occur if the ethanol solution were ingested in smaller amounts throughout the day or accompanied by food. Also, in Abel and Greizerstein's (21) design, ethanol administration was begun on day 5 of gestation, whereas in humans, alcoholism is typically a chronic condition. For these reasons, animal experiments which more closely simulate the human alcoholic condition may be more realistic in the study of FAS.
Numerous workers have administered ethanol in liquid diets. Henderson and Schenker (17) fed female rats a liquid diet of 6% ethanol for 4-21 weeks prior to and throughout gestation. When compared to pair-fed animals, these rats showed no differences in litter size or gestational length. However, the ethanol-fed dams had pups which weighed significantly less than the pups of pair-fed dams.

Several problems may arise from the use of liquid diets. Firstly, Oisund et al. (20) found that liquid diets caused an abnormally large intake of water, the effects of which are unknown. Also, Weiner et al. (27) suggested that most commercially available liquid diets are not nutritionally adequate to meet the needs of pregnant rats.

Animal studies in which ethanol solutions are offered as the only fluid source are known to decrease food consumption (29) and to cause moderate dehydration (30). However, these situations may also occur in the human alcoholic, as alcohol replaces a certain percentage of required calories, and ethanol is a known diuretic. Therefore, an experimental design which provides rats with ad libitum food and an ethanol solution ad libitum may most closely resemble the human situation.

Studies Examining the Mechanisms by which Alcohol Causes FAS

Since the establishment of FAS characteristics in animal models, an increasing number of workers have sought to determine the mechanism by which alcohol impairs fetal growth. The growth retarding effect of maternal alcohol ingestion may be due to a direct action of ethanol or its metabolites upon the fetus, reduced maternal food intake, interference of alcohol with maternal nutrient absorption, increased maternal nutrient loss, reduced blood nutrient levels during pregnancy, or interference with placental nutrient transfer.
1) The Direct Effect of Ethanol

Brown et al. (32) kept 9½-day old rat embryos for 48 hours (the period of organogenesis) in cultures containing 150 mg. or 300 mg. ethanol per 100 ml., or control cultures containing Hanks basic buffered salt solution. Differentiation, growth, total DNA and total protein content were significantly reduced in the 300 mg. alcohol group. Growth measures for the yolk sac and placenta were not affected, nor were gross alterations in morphogenesis seen. In addition, the ratios of total DNA to total protein were not affected significantly by ethanol treatment. Thus, cell number, but not cell size was reduced. Although these findings seem to suggest that fetal growth retardation is a direct result of ethanol toxicity, the mechanism of ethanol's action was not indicated. The possibility still exists that ethanol in the culture medium was interfering with the fetuses' nutrient supply or utilization. Also, the findings of Little et al. (14) (indicating increased incidence of low birth weight babies born to alcoholics who have abstained during pregnancy) suggest that alcohol may have a lasting effect upon maternal metabolism or physiological state.

2) The Effect of Reduced Maternal Food Intake

Pair-feeding studies have shown that the fetal and neonatal growth retardation seen in rats and mice is not simply the result of the reduced food intake of alcohol-treated dams (16-18, 21-23). Studies where offspring were surrogate-fostered after birth, have shown that poor postnatal growth is not a result of reduced maternal care (18).

3) The Effect of Ethanol upon Hormonal Function

Root et al. (33) studied hypothalamic-pituitary function in four
FAS children. Serum levels of human growth hormone, insulin, luteinizing hormone, follicle-stimulating hormone, testosterone and parathyroid hormone were all within normal ranges. The researchers concluded that the postnatal growth retardation of FAS was not a result of abnormal hypothalamic-pituitary function.

4) The Effect of Ethanol upon Placental Nutrient Transfer

Studies on the effect of ethanol upon placental nutrient transfer using rats have produced conflicting results. Lin (34) reported a reduced transfer of alpha-amino-isobutyric acid to fetuses of rats administered alcohol from day 6 to day 21 of gestation. Henderson et al. (35) found a reduced in vitro uptake of valine by placental villous fragments taken from rats which had been intubated with ethanol on days 11-13 or 14-16 of gestation. Henderson et al. (35) also administered 6% ethanol in a liquid diet to rats for 30 days prior to mating and throughout gestation. This chronic ethanol consumption induced a 44% depression in in vitro placental valine uptake tested on day 20 of gestation.

However, in our laboratory, Jones et al. (28) found that rats given ethanol in their drinking water for 5 weeks prior to mating and throughout gestation, did not differ from controls in fetal uptake of zinc, a glucose analog or 2-amino isobutyrate. The lack of agreement between studies may be due to differences in experimental design. The experiment of Lin (34) involved only short term ethanol administration. Henderson et al. (35) used an in vitro placental preparation, whereas Jones et al. (28) fed ethanol to their rats over an extended time period and studied in vivo fetal nutrient uptake.

The findings of Jones et al. (28,30) may suggest that rats chronically
fed ethanol are able to maintain normal nutrient levels, and that the uptake of nutrients by the fetus was not altered. Jones et al. (30) reported that alcohol ingestion reduced maternal blood flow to placentae, but also that alcohol-treated rats had significantly larger placentae when compared to both pair-fed and control animals. Weiner et al. (27) also have reported higher placental weights in rats fed ethanol in a liquid diet. Jones et al. (30) suggested that larger placentae may develop in alcohol-treated dams to compensate for reduced blood flow and to maintain adequate fetal nutriture.

5) The Effect of Ethanol upon Maternal Intestinal Nutrient Absorption

Studies have been done with humans and animals on the effects of alcohol consumption upon the absorption of nutrients from the intestine. In experiments with nonpregnant rats, researchers have reported that alcohol consumption increases fecal zinc loss (36), and decreases intestinal amino acid active transport (37). Increased fecal loss of zinc has also been found in human alcoholic subjects (38).

Lucas et al. (39) studied folic acid transfer across everted jejunal sacs taken from nonpregnant rats. Jejunum which had been taken from rats given a 20% ethanol solution for 3 weeks showed significantly elevated rates of folic acid transfer. However, when jejunum was taken from rats which had not been treated with alcohol, and put directly in a 3% ethanol solution, a decrease in folate transfer resulted.

The interaction of the effects of alcohol consumption, nutritional status and impaired absorption of essential nutrients may be very complex. Alcohol consumption impairs the absorption of some nutrients, which in turn may affect the absorption of other nutrients. Hoyumpa et al. (40)
found that jejunal sacs taken from thiamine deficient rats showed an increased transfer of (14C)-D-glucose when compared to sacs taken from pair-fed animals. However, Hoyumpa et al. also found that a single dose of ethanol by gavage reduced glucose transfer in sacs taken from both thiamine deficient and pair-fed rats. In fact, ethanol decreased glucose transfer in the thiamine deficient group to below that of the pair-fed group.

6) The Effect of Ethanol upon Maternal Urinary Loss of Nutrients

   Alcohol is a known diuretic. Increased urinary loss of zinc by ethanol-fed male rats has been observed (36). Several human studies have shown that alcoholics have increased urinary loss of zinc (38,41,42) and of magnesium (43).

7) The Effect of Ethanol upon Blood and Tissue Nutrient Levels

   a) Zinc

   Decreased tissue zinc levels have been found after 4 weeks of feeding male rats a liquid diet containing 5% ethanol (36). Decreased plasma and liver zinc levels have been reported in male rats given a 20% ethanol solution (44). Also, depressed serum zinc levels were present in 6 of the 10 human alcoholics studied by Sullivan and Heaney (42).

   b) Folic Acid

   Halsted (45) reported that chronic alcoholism in humans is associated with a low dietary folate intake and decreased saturation of plasma B-globulin with folic acid.

   Frank and Baker (46) fed 6 male rats Purina Rat Chow and intubated them daily for 28 days with 6 grams ethanol/kg. body weight. Blood and
liver samples taken from these animals were compared to those of controls maintained on the same chow diet and intubated daily with sucrose solution. Blood folate levels of the ethanol-treated group were significantly lower than those of the controls (871 ng. total folate/ml. blood for ethanol-treated animals and 1086 ng./ml. blood for control animals). However, liver folate levels were increased in the ethanol-treated group (117 ug./gram liver protein) when compared to the controls (80 ug./gram liver protein).

c) Glucose

Several groups of investigators have reported that ethanol inhibits gluconeogenesis (47,48). Krebs et al. (48) used perfused rat livers (from rats fasted for 48 hours) and reported that 10 mmole ethanol caused a 66% reduction of gluconeogenesis from lactate, while 44 mmole ethanol caused a 33% reduction. They proposed that these findings explain the hypoglycemia found in malnourished alcoholics.

The possibility that ethanol has an effect upon the glucose status of pregnant and neonatal rats has been studied by Snyder and Singh (49). They gave rats a liquid diet containing ethanol as 36% of total calories throughout gestation. Maternal blood glucose concentration in the ethanol group and blood glucose of their 2 day old pups were compared to blood glucose from pair-fed and ad libitum fed control animals. Although maternal blood glucose values for ethanol, pair-fed and control groups respectively, were similar (45 mg./dl., 43 mg./dl. and 46 mg./dl.), the blood glucose levels of the 2 day old pups were significantly different from each other (51 mg./dl., 67 mg./dl. and 79 mg./dl. blood for ethanol-treated, pair-fed and control groups respectively). Pups of alcohol-treated dams weighed 12% less than those of pair-fed dams, and 22% less
than those of control dams.

d) Amino Acids

Recently, investigators have studied the effect of ethanol ingestion upon plasma amino acid patterns and upon the incorporation of free amino acids into tissue proteins. Shaw and Lieber (50) studied alcoholics who did not have liver disease and found that they had increased plasma levels of alpha-amino-n-butyric acid.

Eriksson et al. (51) reported that a single intraperitoneal injection of 2 grams ethanol/kg. body weight (20% ethanol w/v) to male rats decreased the plasma levels of all amino acids, with the exception of glutamine and glutamate. Furthermore, the ratio of some amino acids to the total plasma amino acid pool were changed. The relative amounts of alanine, asparagine, phenylalanine and threonine were decreased; the relative amounts of glutamate, glutamine, lysine and ornithine were increased. Stanko et al. (52) reported that a liquid diet containing 34% ethanol administered to rats for 30 days produced a significant increase in plasma leucine, isoleucine, and alpha-amino-n-butyric acid, and a significant decrease in plasma alanine.

The effect of ethanol upon incorporation of amino acids into tissue proteins has also been studied. Several studies (53-55) have shown that ethanol ingestion by adult rodents reduces in vivo hepatic amino acid uptake. Morland et al. (55) reported that hepatic valine uptake was reduced both in male rats fed a low protein diet and in male rats fed a high protein diet. In addition, maternal alcohol consumption has been shown to decrease incorporation of $^{14}$C leucine into fetal brain and hepatic proteins (56-58).
The significance of changes in plasma amino acid pattern and protein synthesis to fetal growth is unknown. However, studies using human subjects have looked at the relationship of maternal and fetal plasma amino acids to fetal growth and development. Moghissi et al. (59) studied the correlation between maternal plasma amino acids (at 32-34 weeks gestation) and newborn development. They found that maternal glycine, lysine, and total amino acids were positively correlated with birth weight, while maternal valine and threonine showed a negative correlation. Barrett et al. (60) reported that cord blood taken from small-for-gestational-age newborns had increased levels of leucine, valine, alanine, cystine, proline, methionine, serine, glycine and lysine, when compared to normal birth weight babies. Scott et al. (61) found that in small-for-gestational-age babies plasma branched-chain amino acids (at day 21 postpartum) correlated inversely with growth and nitrogen retention.

A long-lasting effect of prenatal malnutrition on amino acid patterns has been shown in animal studies. Hsu et al. (62) studied 9 month old male offspring of underfed dams. They found that these animals had decreased levels of all plasma amino acids, even though they had been fed ad libitum since weaning. The most marked decreases were of alanine, glutamic acid, glycine, proline, arginine and valine.

The findings of ethanol-induced decreased total plasma amino acid concentrations may be due to a number of factors. Ethanol may interfere with the intestinal absorption of some amino acids (37); reduce the uptake and incorporation of some amino acids into liver proteins, or extrahepatic tissues (53-58,63); reduce utilization of plasma amino acids for gluconeogenesis (64); increase proteolysis in extrahepatic
tissues, particularly muscle tissue (65,66); or decrease both urea and albumin synthesis (64,67,68)?

**SUMMARY**

Although it has been known for many years that maternal alcohol consumption has deleterious effects upon fetal development, the mechanism of ethanol's action is still unknown. The possibility exists that ethanol interferes with maternal and/or fetal nutrient status. Studies using nonpregnant subjects, have shown that alcohol increases urinary loss of minerals, inhibits hepatic gluconeogenesis, reduces intestinal absorption of nutrients and produces changes in plasma nutrient concentrations. Thus, the present study was designed to examine the effect of maternal alcohol consumption upon plasma levels of several nutrients in rat dams and their fetuses.
EXPERIMENTAL DESIGN AND METHODOLOGY

Treatment of Animals

Forty virgin female Sprague-Dawley rats were obtained from Biobreeding Laboratories of Canada Ltd. (Ottawa, Canada) and housed individually in stainless steel screen-bottom cages. On arrival, the rats weighed between 200 and 220 grams. Throughout the experiment they were kept in a room maintained at 21°C with a 12-hour on, 12-hour off lighting cycle.

After a one week adjustment period, each rat was randomly assigned to one of three dietary regimens. Group 1 ( Alcohol) received 10% (v/v) ethanol in their drinking water and a nutritionally adequate solid diet (Purina Rat Chow) ad libitum. Group 2 (Pair-fed) received the same amount of Purina Rat Chow as Group 1 had consumed in the previous 24 hours, mixed with an amount of cornstarch calorically equivalent to the amount of alcohol consumed by Group 1. The pair-fed group received water ad libitum. To enable easy management, the rat chow was ground fine prior to weighing. Spillage of the ground chow by both the alcohol and pair-fed groups was measured and the food given to the pair-fed animals adjusted accordingly. Group 3 (Control) received Purina Rat Chow and water ad libitum throughout the study. Animals were fed daily between 0830 and 1030.

After one week, the alcohol given to Group 1 animals was increased to 20% (v/v). The animals in each group were maintained on their respective feeding regimens for 5 weeks, after which mating of each animal to male Sprague-Dawley rats was begun. Males and females were housed together overnight. The presence of sperm in the vaginal washings the following morning was taken as day 1 of pregnancy.
Starting on day 1 of gestation the alcohol-treated rats were changed to a regimen of 30% (v/v) ethanol. Pregnant Group 2 animals were pair-fed according to the intake of pregnant alcohol-treated animals at the same stage of gestation. The animals were kept on their respective diets until day 21 of gestation.

Throughout the study weekly body weights of all animals were recorded. Daily food, alcohol and starch consumption by alcohol-treated and pair-fed animals were recorded. No record of food consumption by the control animals was kept. Food consumption by control animals has been recorded in several similar studies in this laboratory (23,26,29, 31), and it was not deemed necessary to repeat the measurements.

Collection and Preparation of Animal Tissues

Between 0800 and 1030 on day 21 of gestation each rat was anesthetized with ether. The chest cavity was opened and approximately 10 ml. of blood was taken by cardiac puncture. The animals were then killed by cutting the heart in two. The duodenum (from the pyloric sphincter to the ligament of Treitz) was removed, rinsed with 10 ml. of saline solution, weighed, and the mucosa removed by scraping with a glass slide. Each sample of mucosa was weighed, and immediately homogenized with 19 parts of distilled water. Half of each homogenate was used to determine conjugase activity, using the radioactive method of Krumdieck and Baugh (69). The remaining sample was frozen at -20°C for later determination of total protein.

From each rat all fetuses and placentae were removed. Each fetus was carefully separated from its placenta and amniotic sac. Umbilical cords were tied off with thread close to the fetus, to prevent loss of fetal blood. Fetuses and placentae from each litter were weighed and an
average weight calculated. Blood was collected from each fetus by cardiac puncture, using drawn glass acid-washed capillary tubes. From 85 to 225 ul. blood was taken from each fetus.

All blood samples were immediately centrifuged in refrigerated centrifuges at 3000 rpm. Plasma was collected and stored in acid-washed plastic tubes. Plasma from fetuses of the same litter was pooled. Plasma to be used for zinc, magnesium, glucose, folic acid, albumin, lactic acid and osmolality determinations was immediately frozen and stored at -70°C until the time of analysis. A portion of maternal plasma for amino acid analysis was deproteinized with 0.1 parts of 50% sulfosalicylic acid. Fetal plasma for amino acid analysis was deproteinized with 3 parts of 2% sulfosalicylic acid. These dilutions were established during pretrials to obtain the correct pH and amino acid concentration for accurate analysis. The samples for amino acid analysis were then stored at -70°C. All plasma nutrient determinations were done within 1 to 5 months of the time of sample collection.

Assay Procedures

Determination of plasma glucose, folic acid, zinc, magnesium and free amino acids was done on both fetal and maternal samples. Plasma osmolality and albumin levels were determined only in maternal samples. Plasma lactic acid concentrations were determined only in fetal samples. Plasma glucose, lactic acid, albumin and osmolality, and maternal intestinal protein and conjugase activity were determined by the author. Plasma amino acid levels were analyzed by lab technicians of the Children's Hospital, Vancouver B.C. Mrs. S. Horsky of the U.B.C. Geological Sciences department did the zinc and magnesium analyses. Plasma folic
acid assays were carried out by Dr. P. Cornwell (Department of Nutrition Sciences, the University of Alabama in Birmingham).

Plasma free amino acids were analyzed by ion-exchange chromatography using a Durrum (Dionex) D-500 amino acid analyzer. Each amino acid was eluted on an ion-exchange column, then reacted with ninhydrin to produce a purple colour. Optical density of the colour was then measured spectrophotometrically.

Plasma glucose was determined using the glucose oxidase method (Sigma Chemical Company, Technical Bulletin No. 510). Maternal plasma albumin levels were determined by a colorimetric assay (Sigma Chemical Company, Technical Bulletin No. 630). Fetal lactic acid determination was performed using the lactic acid procedure (Sigma Chemical Company, Technical Bulletin No. 826-UV).

Plasma zinc and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer Atomic Absorption Spectrophotometer, Model 603).

Plasma folic acid was determined microbiologically with *Lactobacillus casei*. This method is based on the observation that certain microorganisms require specific vitamins for growth. Using a basal medium complete in all respects except for the vitamin under test, growth responses of the organism are compared quantitatively in standard and unknown solutions. Optical density of the culture medium at 650 nm is used as a measure of growth and folic acid content (70).

Maternal intestinal conjugase (pteroyl-poly-γ-glutamyl hydrolase) activity was determined using the method of Krumdieck and Baugh (69). The assay is based upon the release and quantitation of radioactive glutamic acid from labeled pteroyldι-γ-glutamyl-(U-C\textsuperscript{14}-glutamic) acid
of known specific radioactivity. At the end of an incubation period, the unreacted substrate is removed by adsorption onto charcoal. The nonadsorbed radioactive glutamic acid is quantitated in a liquid scintillation counter. Conjugase activity is then expressed as counts per minute per mg. protein.

Total protein of intestinal mucosa samples was assayed by the method of Lowry et al. (71), using bovine serum albumin as a standard.

To investigate the possibility that alcohol ingestion results in dehydration, plasma osmolality was measured in maternal samples, using an osmometer (Precision Systems Inc., Newton, Mass.).

**Statistical Analysis:** Differences in plasma nutrient levels among the three experimental groups were examined using the Midas Computer Package analysis of variance. One way analysis of variance was used. In the case of significant differences, Scheffe Allowances (0.95 and 0.99) with paired comparisons were used to identify differences among treatment groups.

Analysis of the plasma nutrient data was also done using Pearson Correlation Coefficients (Statistical Package for the Social Sciences). Average fetal weight (alcohol-treated, pair-fed and control fetuses combined) was examined for correlation to 62 separate variables. These variables included maternal prepregnancy weight gain; maternal weight gain throughout pregnancy; placental weight; maternal plasma levels of folic acid, zinc, magnesium, glucose, albumin and 26 amino acids; and fetal plasma levels of folic acid, zinc, magnesium, glucose and 25 amino acids. Each correlation which showed statistical significance (p<0.05) was examined using a scattergram (Statistical Package for the Social
Sciences). This was done to eliminate the possibility that significant correlations were found between data occurring in clumps.

Differences in maternal body weight among the three groups (both prior to and throughout gestation) were also analyzed using the Midas one-way analysis of variance. Data on food intake and alcohol consumption were analyzed by repeated measures analysis of variance (Midas *ANOVAR) for the factors of diet and time.
RESULTS

Caloric Intakes and Maternal Body Weights

Table 1 shows the average daily food and alcohol consumption of rats given alcohol or pair-fed prior to pregnancy. Throughout days 1-7 alcohol-treated animals were given a drinking solution of 10% alcohol. On day 8 this was increased to 20% ethanol. This increase in ethanol concentration almost doubled the mean daily absolute alcohol intake (p<0.05) of alcohol-treated animals, and produced a slight, but not significant, reduction in their mean daily food consumption. Prior to pregnancy, mean daily food consumption of alcohol-treated (and therefore also of pair-fed) animals only increased significantly (p<0.05) between weeks 3 and 4 of the experiment.

In Table 2 average food and alcohol consumption prior to pregnancy are expressed in calories. During this period, the mean daily caloric intake of alcohol-treated and pair-fed animals was 52.39±5.81 kcal. On average, alcohol or starch consumption accounted for 23.25±5.27% of total daily calories. Percent of calories from alcohol or starch consumption during days 1-7 was 14.20%, which increased to 24.70% during days 8-14 when the alcohol concentration of the drinking solution was increased from 10% to 20%.

When food consumption was expressed as grams/gram body weight, it was found that prior to pregnancy, alcohol-treated and pair-fed animals consumed from 58 to 65% of the food consumed by control animals in a similar study by Jones et al. (28). However, expressed as kcal/gram body weight, total daily caloric intake of alcohol-treated and pair-fed animals prior to pregnancy in the present study was 70 to 92% of the
daily caloric intake of control rats reported by Jones et al. (28).

Table 3 shows the average daily food and alcohol consumption of alcohol-treated and pair-fed animals throughout pregnancy. On day 1 of pregnancy the concentration of ethanol in drinking water given to alcohol-treated animals was increased to 30%. This increase did not produce an increase in alcohol consumption of alcohol-treated animals during the first week of pregnancy. Mean daily food consumption of alcohol-treated animals during the first week of pregnancy decreased slightly when compared to the week just prior to pregnancy (however, due to the large standard deviations, this decrease was not significant). Throughout pregnancy mean daily food consumption of alcohol-treated animals increased, although this increase was significant (p<0.05) only between weeks 1 and 2 of gestation. Mean daily alcohol intake increased significantly only between weeks 2 and 3. Mean alcohol intake during the third week of gestation was 7.5±3.27 ml. Fluid intake by pregnant rats has been shown to increase during the final days of pregnancy. However, other studies done in this laboratory (22,28) have not reported as large an increase in alcohol consumption as was found in the present study.

The total caloric intake of alcohol-treated and pair-fed dams during pregnancy is presented in Table 4. The mean daily energy intake of both groups was 40 kcal. higher during the last week of gestation, when compared to the first week. This was accompanied by an increase of 14% in the number of daily calories derived from alcohol or starch. During gestation calories derived from alcohol or starch consumption accounted for an average of 35.35±7.82% of total calories.

During the first week of pregnancy, alcohol-treated dams consumed
52% and pair-fed dams consumed 47% (grams/gram body weight) of the food consumed by controls reported by Jones et al. (28). This percentage increased throughout gestation. Chow consumed by alcohol-treated dams was 74 and 76% of that consumed by control dams (Jones et al.) for the second and third weeks of gestation, while chow consumed by pair-fed dams was 67 and 63% of that of control dams (Jones et al.) for the second and third weeks of gestation. Differences in percent of chow consumed between alcohol-treated and pair-fed dams were due to the greater body weight of pair-fed animals.

Mean daily caloric intake expressed a kcal./gram body weight, of alcohol-treated dams was 69%, 100% and 115% of that of control dams (Jones et al., 28) for the first, second and third weeks of gestation respectively. Mean daily caloric intake of pair-fed dams was 63%, 93% and 96% of that of control dams (Jones et al., 28) for the first, second and third weeks of gestation.

Table 5 and Figures 1 and 2 show the average body weights of alcohol-treated, pair-fed and control animals throughout the experiment. Except for initial weights, the body weights of control animals were significantly greater (p<0.05) than those of the other two experimental groups throughout the entire experiment.

During the 5 weeks prior to pregnancy there were no significant differences in body weight between alcohol-treated and pair-fed animals. The body weights of both alcohol-treated and pair-fed animals dropped slightly during the first week of the experiment. This was probably due to adjustment by alcohol-treated animals to the first introduction of ethanol into their drinking water. After the first week, body weight of both alcohol-treated and pair-fed animals showed a steady increase.
During pregnancy, statistically significant differences in body weight were seen among all three treatment groups. Body weight of control animals was considerably greater than that of both alcohol-treated (p < 0.001), and pair-fed (p < 0.05) animals. During the last two weeks of pregnancy, the body weights of the pair-fed animals were greater than those of the alcohol-treated dams (p < 0.005). This was probably due to the initial weight loss by alcohol-treated animals during the first week of pregnancy. This weight loss may have been due to the regimen of drinking water containing 30% ethanol which was initiated on day 1 of pregnancy. Weight loss by alcohol-treated animals was not paralleled by a similar weight loss by pair-fed animals. During the last week of pregnancy both pair-fed and control groups gained an average of 60 grams, while alcohol-treated animals gained only an average of 30 grams.

No correlation was found between fetal body weight (alcohol-treated, pair-fed and control fetuses combined) and maternal prepregnancy weight gain (alcohol-treated, pair-fed and control dams combined). However, a Pearson correlation coefficient of 0.6473 (p < 0.001) was found between fetal body weight and maternal weight gain throughout pregnancy.

**Litter Size, Fetal Body Weights and Placental Weights**

Table 6 shows mean litter size, fetal body weight and placental weight determined on day 21 of gestation. Means and standard deviations were calculated using litter means. Mean fetal body weight was significantly lower (p < 0.001) in the alcohol-treated group compared to the pair-fed or control groups. There was not a significant difference in body weight between pair-fed and control fetuses.

Mean litter size (number of fetuses per litter) did not vary
significantly among the three groups.

Placental weights were highest in the alcohol-treated group, and lowest in the pair-fed group ($p<0.05$). However, there was not a significant difference in placental weight between the alcohol-treated and control groups. No significant correlation existed between fetal body weight and placental weight.
**Table 1** Daily Food and Alcohol Consumption Prior to Pregnancy
(Mean ± S.D.)

<table>
<thead>
<tr>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>food(g)</td>
<td>food(g)</td>
</tr>
<tr>
<td>alcohol(ml)</td>
<td>starch(g)</td>
</tr>
</tbody>
</table>

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of rats</strong></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Prepregnancy Days</strong></th>
<th><strong>1-7</strong></th>
<th><strong>8-14</strong></th>
<th><strong>15-21</strong></th>
<th><strong>22-28</strong></th>
<th><strong>29-35</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>9.39(a)</td>
<td>1.20(a)</td>
<td>9.39(a)</td>
<td>1.65(a)</td>
<td>9.39(a)</td>
</tr>
<tr>
<td>±1.61</td>
<td>±0.22</td>
<td>±0.83</td>
<td>±0.52</td>
<td>±0.96</td>
<td>±1.65</td>
</tr>
<tr>
<td>8-14</td>
<td>8.29(a)</td>
<td>2.10(b)</td>
<td>8.29(a)</td>
<td>2.89(b)</td>
<td>8.29(a)</td>
</tr>
<tr>
<td>±0.83</td>
<td>±0.52</td>
<td>±0.96</td>
<td>±0.38</td>
<td>±1.06</td>
<td>±0.83</td>
</tr>
<tr>
<td>15-21</td>
<td>8.89(a)</td>
<td>2.66(b)</td>
<td>8.89(a)</td>
<td>3.66(b)</td>
<td>8.89(a)</td>
</tr>
<tr>
<td>±0.96</td>
<td>±0.38</td>
<td>±0.96</td>
<td>±0.38</td>
<td>±1.06</td>
<td>±0.96</td>
</tr>
<tr>
<td>22-28</td>
<td>10.09(b)</td>
<td>2.57(b)</td>
<td>10.09(b)</td>
<td>3.53(b)</td>
<td>10.09(b)</td>
</tr>
<tr>
<td>±0.56</td>
<td>±0.26</td>
<td>±0.56</td>
<td>±0.26</td>
<td>±1.06</td>
<td>±0.56</td>
</tr>
<tr>
<td>29-35</td>
<td>10.40(b)</td>
<td>2.74(b)</td>
<td>10.40(b)</td>
<td>3.77(b)</td>
<td>10.40(b)</td>
</tr>
<tr>
<td>±0.69</td>
<td>±0.28</td>
<td>±0.69</td>
<td>±0.28</td>
<td>±1.06</td>
<td>±0.69</td>
</tr>
<tr>
<td></td>
<td>±0.56</td>
<td>±0.56</td>
<td>±0.56</td>
<td>±1.06</td>
<td>±0.56</td>
</tr>
</tbody>
</table>

a,b Figures in the same column not sharing the same subscript are significantly different at the p<0.05 level.
Table 2  Daily Caloric Intake Prior to Pregnancy (Mean ± S.D.)

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>food (kcal)</td>
<td>alcohol (kcal)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

Prepregnancy Days

<table>
<thead>
<tr>
<th>Days</th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>food (kcal)</td>
<td>alcohol (kcal)</td>
</tr>
<tr>
<td></td>
<td>±6.87</td>
<td>±1.19</td>
</tr>
<tr>
<td>1-7</td>
<td>39.89</td>
<td>6.61</td>
</tr>
<tr>
<td>8-14</td>
<td>35.22</td>
<td>11.55</td>
</tr>
<tr>
<td>15-21</td>
<td>37.77</td>
<td>14.63</td>
</tr>
<tr>
<td>22-28</td>
<td>42.87</td>
<td>14.16</td>
</tr>
<tr>
<td>29-35</td>
<td>44.20</td>
<td>15.05</td>
</tr>
</tbody>
</table>
Table 3  Daily Food and Alcohol Consumption During Pregnancy  
(Mean ± S.D.)

<table>
<thead>
<tr>
<th>Pregnancy Days</th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>food(g)</td>
<td>food(g)</td>
</tr>
<tr>
<td></td>
<td>alcohol(ml)</td>
<td>starch(g)</td>
</tr>
<tr>
<td>No. of rats</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>1-7</td>
<td>9.11(b) ±2.21</td>
<td>9.11(b) ±0.00</td>
</tr>
<tr>
<td></td>
<td>2.91(b) ±0.60</td>
<td>4.00(b) ±0.00</td>
</tr>
<tr>
<td>8-14</td>
<td>11.60(c) ±1.89</td>
<td>11.60(c) ±0.00</td>
</tr>
<tr>
<td></td>
<td>4.34(b) ±1.09</td>
<td>5.97(b) ±0.00</td>
</tr>
<tr>
<td>15-21</td>
<td>12.27(c) ±3.37</td>
<td>12.27(c) ±0.00</td>
</tr>
<tr>
<td></td>
<td>7.50(c) ±3.27</td>
<td>10.31(c) ±0.00</td>
</tr>
</tbody>
</table>

b,c Figures in the same column not sharing the same subscript are significantly different at the p<0.05 level.
Table 4 Daily Caloric Intake During Pregnancy (Mean ± S.D.)

<table>
<thead>
<tr>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>food (kcal)</td>
<td>food (kcal)</td>
</tr>
<tr>
<td>alcohol (kcal)</td>
<td>starch (kcal)</td>
</tr>
<tr>
<td>total daily kcal</td>
<td>total daily kcal</td>
</tr>
<tr>
<td>from alcohol</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>13</th>
<th>13</th>
<th>13</th>
<th>13</th>
<th>10</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7</td>
<td>38.74 ± 9.38</td>
<td>16.03 ± 3.30</td>
<td>54.77 ± 9.38</td>
<td>29.27 ± 9.38</td>
<td>38.74 ± 0.00</td>
<td>16.03 ± 0.00</td>
<td>54.77 ± 0.00</td>
</tr>
<tr>
<td>8-14</td>
<td>49.30 ± 8.01</td>
<td>23.85 ± 6.00</td>
<td>73.15 ± 8.01</td>
<td>32.60 ± 6.00</td>
<td>49.30 ± 0.00</td>
<td>23.85 ± 0.00</td>
<td>73.15 ± 0.00</td>
</tr>
<tr>
<td>15-21</td>
<td>52.14 ± 14.31</td>
<td>41.25 ± 18.00</td>
<td>93.39 ± 14.31</td>
<td>44.17 ± 18.00</td>
<td>52.14 ± 0.00</td>
<td>41.25 ± 0.00</td>
<td>93.39 ± 0.00</td>
</tr>
</tbody>
</table>
Table 5  Weekly Maternal Body Weights Before and During Pregnancy (Mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>13</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Prepregnancy Days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>222.6±9.23(a)</td>
<td>225.5±11.23(a)</td>
<td>226.1±10.16(a)</td>
</tr>
<tr>
<td>Day 7</td>
<td>223.3±15.51(a)</td>
<td>219.3±10.22(a)</td>
<td>231.4±13.45(b)</td>
</tr>
<tr>
<td>Day 14</td>
<td>221.6±17.21(a)</td>
<td>221.2±9.72(a)</td>
<td>242.2±16.21(b)</td>
</tr>
<tr>
<td>Day 21</td>
<td>228.6±17.46(a)</td>
<td>229.2±8.48(a)</td>
<td>251.9±16.32(b)</td>
</tr>
<tr>
<td>Day 28</td>
<td>236.2±16.35(a)</td>
<td>236.3±8.50(a)</td>
<td>256.5±16.69(b)</td>
</tr>
<tr>
<td>Day 35</td>
<td>240.4±19.89(a)</td>
<td>241.7±8.72(a)</td>
<td>261.3±17.01(b)</td>
</tr>
<tr>
<td><strong>Pregnancy Days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>257.4±19.54(a)</td>
<td>260.8±11.46(a)</td>
<td>270.4±21.08(b)</td>
</tr>
<tr>
<td>Day 7</td>
<td>250.2±23.80(a)</td>
<td>272.5±12.54(b)</td>
<td>293.2±21.79(c)</td>
</tr>
<tr>
<td>Day 14</td>
<td>270.5±28.28(a)</td>
<td>297.6±10.97(b)</td>
<td>313.6±21.70(c)</td>
</tr>
<tr>
<td>Day 21</td>
<td>298.2±36.41(a)</td>
<td>358.0±17.83(b)</td>
<td>378.2±25.49(c)</td>
</tr>
</tbody>
</table>

a,b,c Figures in the same row not sharing the same subscript are significantly different at least at the p<0.05 level.
Figure 1. Mean Maternal Body Weight Prior to Pregnancy

- Alcohol-treated
- Pair-fed
- Control
Figure 2: Mean Maternal Body Weight During Pregnancy

- Alcohol-treated
- Pair-fed
- Control
Table 6  Fetal Body Weight, Litter Size and Placental Weight
(Mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of litters</td>
<td>13</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Fetal body weight (g)</td>
<td>2.89±0.32(a)</td>
<td>3.40±0.30(b)</td>
<td>3.49±0.39(b)</td>
</tr>
<tr>
<td>Litter size</td>
<td>10.0±3.58</td>
<td>11.6±1.71</td>
<td>10.3±1.95</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>0.53±0.09(a)</td>
<td>0.46±0.04(b)</td>
<td>0.50±0.05(a)</td>
</tr>
</tbody>
</table>

a, b Figures in the same row not sharing the same subscript are significantly different at least at the p<0.05 level.
Nutrient Levels in Maternal Plasma

Mean plasma levels of folic acid, zinc, magnesium, glucose and albumin in rat dams at day 21 of gestation are presented in Table 7.

1) Folic Acid

No differences were observed in mean plasma folic acid levels among the treatment groups. A significant correlation was not found between fetal body weight and maternal plasma folic acid levels.

2) Zinc

There were no significant differences found in plasma zinc concentration among the three groups of dams, nor was there a significant correlation between fetal body weight and maternal plasma zinc levels.

3) Magnesium

Mean maternal plasma magnesium levels were significantly higher ($p < 0.01$) in alcohol-treated dams when compared to both pair-fed and control animals, but there was not a significant difference between pair-fed and control samples. No correlation was found between maternal plasma magnesium concentration and fetal body weight.

4) Glucose

There were no significant differences among the three maternal treatment groups in plasma glucose concentration. Pearson correlation showed that no significant correlation existed between maternal plasma glucose values and fetal body weight.

5) Albumin

The three groups of dams had similar plasma levels of albumin on day 21 of gestation. No correlation was found between maternal plasma...
albumin levels and fetal body weight.

**Maternal Plasma Osmolality**

Mean plasma osmolality in rat dams is shown in Table 8. Osmolality was highest in alcohol-treated dams and lowest in pair-fed dams. Differences in degree of dehydration between alcohol-treated and pair-fed dams and between alcohol-treated and control dams were significant at the p<0.01 level. The difference in plasma osmolality between pair-fed and control animals was not significant.

**Maternal Intestinal Conjugase Activity**

Intestinal conjugase activity is presented in Table 9. Pteroyl-poly-γ-glutamyl hydrolase activity in maternal duodenal mucosa was expressed as counts per minute per mg. mucosal protein. No significant differences were found among the three groups of animals.
Table 7  Maternal Plasma Folate, Zinc, Magnesium, Glucose and Albumin Levels on Day 21 of Gestation (Mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid (ng/ml)</td>
<td>4.99±6.85(4)a</td>
<td>6.69±2.00(4)</td>
<td>12.50±6.61(4)</td>
</tr>
<tr>
<td>Zinc (mg/ml)</td>
<td>0.96±0.27(7)</td>
<td>1.06±0.36(7)</td>
<td>1.18±0.29(8)</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>3.40±0.86(7)b</td>
<td>2.17±0.39(7)</td>
<td>2.20±0.43(8)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>129.11±26.23(13)</td>
<td>117.14±20.29(7)</td>
<td>122.08±21.77(13)</td>
</tr>
<tr>
<td>Albumin (ug/ml)</td>
<td>3.71±0.51(7)</td>
<td>3.28±0.31(7)</td>
<td>3.40±0.33(8)</td>
</tr>
</tbody>
</table>

a Numbers in brackets represent the number of animals.
b Significantly different (p<0.01) from both pair-fed and control values.
**Table 8** Maternal Plasma Osmolality on Day 21 of Gestation (Mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Osmolality (mosmoles/l)</td>
<td>296.57±12.25a</td>
<td>267.57±16.79</td>
<td>273.38±8.23</td>
</tr>
</tbody>
</table>

*a* Significantly different at p<0.01 from both pair-fed and control.

**Table 9** Maternal Intestinal Conjugase Activity on Day 21 of Gestation (Mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>9</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Conjugase Activity</td>
<td>48967±15549a</td>
<td>40276±3736</td>
<td>61355±16673</td>
</tr>
<tr>
<td>(CPM/mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* There were no significant differences among the three groups.
Maternal Plasma Amino Acids

The mean levels of maternal plasma amino acids and urea at day 21 of gestation are shown in Table 10. Levels of maternal plasma amino acids are also illustrated in Figure 3. In control animals mean plasma amino acid levels varied from the lowest value of 14.17 umoles/liter for alpha-amino-n-butyric acid, to the highest value of 976.00 umoles/liter for lysine. Generally, mean amino acid levels in alcohol-treated and pair-fed animals were similar to those in control animals. However, within each of the three groups, there was wide variation in amino acid levels. Only seven out of the twenty-five amino acids examined showed statistically significant differences among the three treatment groups. These seven amino acids were threonine, serine, proline, alpha-amino-n-butyric acid, cystine, alanine and histidine.

Of these seven amino acids only proline and alpha-amino-n-butyric acid were significantly different in alcohol-treated dams compared to both pair-fed and control dams. Mean proline levels were significantly lower (p<0.05), while mean plasma alpha-amino-n-butyric acid levels were significantly higher (p<0.01) in alcohol-treated dams compared to the other two groups.

Mean plasma threonine levels were significantly lower in pair-fed (310.43 umoles/l.) when compared to control dams (419.00 umoles/l.), while the levels in alcohol-treated and control dams were similar. Mean plasma serine and alanine levels in pair-fed animals were significantly higher (p<0.01) than those in both alcohol-treated and control groups.

Plasma levels of cystine and histidine were similar in alcohol-treated and pair-fed animals. Cystine levels were significantly lower in the alcohol-treated compared to the control group (p<0.01), and in the pair-
fed compared to the control group \((p<0.05)\). Control females exhibited significantly higher histidine levels than alcohol-treated \((p<0.05)\), and pair-fed females \((p<0.01)\).

Mean plasma levels of urea varied greatly among all three treatment groups. Pair-fed animals exhibited significantly lower urea levels than both alcohol-treated and control animals \((p<0.01)\). Urea levels in alcohol-treated animals were significantly lower than those of control animals \((p<0.05)\).

Two of the maternal plasma amino acids showed significant correlations with fetal body weight. A positive correlation \((0.5694, p<0.05)\) was found between maternal plasma proline levels and fetal body weight. A negative correlation of \(-0.6409 (p<0.01)\) was found between maternal plasma alpha-amino-n-butyric acid levels and fetal body weight.
<table>
<thead>
<tr>
<th>Amino Acids (umol/l)</th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Alanine</td>
<td>621.83±75.72a</td>
<td>949.57±126.02b</td>
<td>612.33±112.74a</td>
</tr>
<tr>
<td>ANBA</td>
<td>46.50±7.50a</td>
<td>9.00±1.00b</td>
<td>14.17±3.87b</td>
</tr>
<tr>
<td>Arginine</td>
<td>122.00±34.69</td>
<td>124.14±14.88</td>
<td>147.33±53.28</td>
</tr>
<tr>
<td>Asparagine</td>
<td>110.00±10.24</td>
<td>100.86±18.31</td>
<td>104.83±22.34</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>18.83±2.32</td>
<td>22.29±2.98</td>
<td>18.83±4.58</td>
</tr>
<tr>
<td>Citrulline</td>
<td>50.83±8.84</td>
<td>71.00±11.48</td>
<td>66.50±19.61</td>
</tr>
<tr>
<td>Cystine</td>
<td>34.17±7.86a</td>
<td>38.86±6.72a</td>
<td>54.33±10.29b</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>135.33±39.22</td>
<td>163.71±26.19</td>
<td>150.00±46.99</td>
</tr>
<tr>
<td>Glutamine</td>
<td>972.00±111.14</td>
<td>702.00±36.77</td>
<td>583.00±0.00</td>
</tr>
<tr>
<td>Glycine</td>
<td>143.83±60.41</td>
<td>102.14±38.62</td>
<td>88.17±20.49</td>
</tr>
<tr>
<td>Histidine</td>
<td>31.33±3.20a</td>
<td>26.29±7.18a</td>
<td>42.33±7.60b</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>21.67±5.89</td>
<td>29.00±7.87</td>
<td>21.33±7.45</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>86.83±19.39</td>
<td>86.57±11.33</td>
<td>97.83±14.33</td>
</tr>
<tr>
<td>Leucine</td>
<td>103.33±31.41</td>
<td>130.71±18.21</td>
<td>151.17±26.45</td>
</tr>
<tr>
<td>Lysine</td>
<td>818.50±161.89</td>
<td>790.57±205.86</td>
<td>976.00±207.58</td>
</tr>
<tr>
<td>Methionine</td>
<td>46.67±10.63</td>
<td>49.29±6.65</td>
<td>51.00±9.34</td>
</tr>
<tr>
<td>Ornithine</td>
<td>56.83±18.85</td>
<td>47.86±9.48</td>
<td>56.17±14.22</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>67.33±10.29</td>
<td>76.00±8.64</td>
<td>69.33±15.49</td>
</tr>
<tr>
<td>Proline</td>
<td>142.83±62.38a</td>
<td>222.57±43.12b</td>
<td>245.50±54.15b</td>
</tr>
<tr>
<td>Serine</td>
<td>253.17±26.14a</td>
<td>327.57±47.60b</td>
<td>227.83±29.90a</td>
</tr>
<tr>
<td>Taurine</td>
<td>192.83±62.77</td>
<td>177.57±45.28</td>
<td>166.50±53.47</td>
</tr>
<tr>
<td></td>
<td>Alcohol Group</td>
<td>Pair-fed Group</td>
<td>Control Group</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------</td>
<td>-------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Threonine</td>
<td>348.17±34.68a;b</td>
<td>310.41±50.96a</td>
<td>419.00±63.45b</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>56.67±18.02</td>
<td>53.43±19.41</td>
<td>76.00±5.66</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>47.83±11.02</td>
<td>47.14±9.51</td>
<td>50.00±16.83</td>
</tr>
<tr>
<td>Valine</td>
<td>159.00±43.62</td>
<td>151.29±22.97</td>
<td>182.50±24.87</td>
</tr>
<tr>
<td>Urea</td>
<td>5316.80±280.83a</td>
<td>2524.00±1081.20b</td>
<td>6850.00±945.37c</td>
</tr>
</tbody>
</table>

a, b, c Figures in the same row not sharing the same subscript are significantly different at least at the p<0.05 level.
Figure 3. Maternal Plasma Amino Acid Levels on Day 21 of Gestation (umoles/liter)

Alanine
Alc 622
PF 950
Cont 612

ANBA
Alc 47
PF 9
Cont 14

Arginine
Alc 122
PF 124
Cont 147

Asparagine
Alc 110
PF 101
Cont 105

Aspartic Acid
Alc 19
PF 22
Cont 19

Cystine
Alc 34
PF 39
Cont 54

Citrulline
Alc 51
PF 71
Cont 67

Glutamic Acid
Alc 135
PF 164
Cont 150

Glutamine
Alc 972
PF 702
Cont 583

Glycine
Alc 144
PF 102
Cont 88
Figure 3 continued

**Histidine**
- Alc 31 ##
- PF 26 ##
- Cont 42 ##

**Hydroxyproline**
- Alc 22 #
- PF 29 ##
- Cont 21 #

**Isoleucine**
- Alc 87 ######
- PF 87 ######
- Cont 98 ######

**Leucine**
- Alc 130 #######
- PF 131 #######
- Cont 151 #######

**Lysine**
- Alc 819 #
- PF 791 #
- Cont 976 #

**Methionine**
- Alc 47 ##
- PF 49 ##
- Cont 51 ##

**Ornithine**
- Alc 57 ##
- PF 48 ##
- Cont 56 ##

**Phenylalanine**
- Alc 67 ####
- PF 76 ####
- Cont 69 ####

**Proline**
- Alc 143 #######
- PF 223 #######
- Cont 246 #######

**Serine**
- Alc 253 #######
- PF 328 #######
- Cont 228 #######
Figure 3 continued

Taurine
   Alc 193
   PF 178
   Cont 167

Threonine
   Alc 348
   PF 310
   Cont 167

Tryptophan
   Alc 57
   PF 53
   Cont 76

Tyrosine
   Alc 48
   PF 47
   Cont 50

Valine
   Alc 159
   PF 151
   Cont 183

(a) # corresponds to 17 umoles of amino acid per liter of plasma
Nutrient Levels in Fetal Plasma

Mean plasma levels of folic acid, zinc, magnesium, glucose, and lactic acid in rat fetuses at day 21 of gestation are presented in Table 11.

1) Folic Acid

No differences in plasma folate were observed among the three fetal groups. There was not a significant correlation between fetal body weight and fetal plasma folic acid levels (alcohol-treated, pair-fed and control fetuses combined).

2) Zinc

Plasma zinc levels were similar in alcohol-treated and pair-fed animals. Mean plasma zinc levels of control fetuses were significantly lower than those of alcohol-treated (p<0.01) and pair-fed (p<0.001) fetuses. No significant correlation was found between fetal body weight and fetal plasma zinc values.

3) Magnesium

Pair-fed fetuses exhibited lower mean plasma magnesium levels than control fetuses (p<0.05). However, there was not a significant difference in plasma magnesium between alcohol-treated and pair-fed, nor between alcohol-treated and control fetuses.

No correlation was found between fetal body weight and fetal plasma magnesium levels.

4) Glucose

Mean plasma glucose levels were higher in both control (p<0.01) and pair-fed (p<0.05) fetuses compared to alcohol-treated fetuses. The Pearson correlation coefficient of 0.6072 (p<0.01) generated for fetal body weight
and fetal plasma glucose, indicated a moderate degree of relationship between the two variables.

5) Lactic Acid

All fetuses exhibited similar mean plasma lactic acid levels. Also, no correlation was found between fetal plasma lactic acid concentration and fetal body weight.
<table>
<thead>
<tr>
<th></th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid (ng/ml)</td>
<td>93.25±12.58(4)a</td>
<td>78.00±17.34 (4)</td>
<td>89.50±15.29(4)</td>
</tr>
<tr>
<td>Zinc (ug/ml)</td>
<td>4.92±0.73(6)b</td>
<td>5.76±1.24(8)b</td>
<td>2.91±0.57(6)c</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>6.30±1.52(6)b,c</td>
<td>5.61±1.56(8)b</td>
<td>7.18±0.45(6)c</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>21.34±9.42(14)b</td>
<td>34.09±10.57(7)c</td>
<td>38.93±11.63(8)c</td>
</tr>
<tr>
<td>Lactic Acid (mg/dl)</td>
<td>194.17±3.82(4)</td>
<td>178.33±7.64(3)</td>
<td>190.00±4.33(3)</td>
</tr>
</tbody>
</table>

a Numbers in brackets represent numbers of litters.
b,c Figures in the same row not sharing the same subscript are significantly different at least at the p<0.05 level.
Fetal Plasma Amino Acids

Fetal plasma levels of amino acids and urea at day 21 of gestation are shown in Table 12. In addition, fetal plasma amino acid levels are illustrated in Figure 4. In control fetuses mean plasma amino acid levels were lowest for cystine (30.17 umoles/liter) and highest for lysine (1476.00 umoles/liter). Generally, mean amino acid levels in alcohol-treated and pair-fed fetuses were similar to those in control fetuses. However, within group variation in amino acid level was wide. Thus, only six of the twenty-four amino acids examined showed statistically significant differences among the three treatment groups. These six amino acids were aspartic acid, threonine, serine, tyrosine, phenylalanine and alanine.

Plasma aspartic acid levels were alike in control and pair-fed fetuses, and higher than those of alcohol-treated fetuses (p<0.05).

The differences in mean plasma tyrosine and threonine concentration were only significant between pair-fed and control fetuses. Mean plasma threonine levels were higher in control compared to pair-fed fetuses (p<0.01). Mean plasma tyrosine levels were lower in control compared to pair-fed fetuses (p<0.05).

Fetal plasma levels of alanine, phenylalanine and serine were similar in alcohol-treated and control animals, but significantly lower than those found in pair-fed animals. Levels of significance for alanine and phenylalanine were p<0.05 for alcohol-treated compared to pair-fed fetuses, and p<0.01 for control compared to pair-fed fetuses. Plasma serine comparisons were significantly different at the p<0.05 level.

Mean plasma histidine levels varied widely among the three fetal groups. However, the standard deviation values for pair-fed and control groups were very large, thus significant differences were not found.
Urea levels were highest in control animals and lowest in pair-fed animals, with alcohol-treated animals exhibiting an intermediate value \( (p<0.01) \).

Significant correlations were found between fetal body weight and two of the 24 amino acids examined. A correlation of \(-0.8279\ (p<0.05)\) occurred between fetal body weight and fetal plasma levels of alpha-amino-\(n\)-butyric acid. A correlation of \(-0.5695\ (p<0.05)\) occurred between fetal body weight and fetal plasma lysine levels.
Table 12  Fetal Plasma Amino Acid and Urea Levels on Day 21 of Gestation
(Mean ± S.D.)

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Alcohol Group</th>
<th>Pair-fed Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of litters 6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Alanine</td>
<td>1304.80±141.30a</td>
<td>1594.30±179.25b</td>
<td>1193.80±108.97a</td>
</tr>
<tr>
<td>ANBA</td>
<td>82.17±21.46</td>
<td>25.00±0.00</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>236.17±27.10</td>
<td>240.86±24.03</td>
<td>232.67±55.61</td>
</tr>
<tr>
<td>Asparagine</td>
<td>190.50±40.36</td>
<td>174.43±31.35</td>
<td>262.67±249.55</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>60.33±6.62a</td>
<td>80.86±17.41b</td>
<td>79.33±7.74b</td>
</tr>
<tr>
<td>Cystine</td>
<td>26.50±10.64</td>
<td>32.86±7.29</td>
<td>30.17±9.37</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>475.00±51.97</td>
<td>558.00±120.15</td>
<td>552.33±82.83</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1491.50±131.53</td>
<td>1352.40±182.09</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>265.00±34.53</td>
<td>317.86±58.16</td>
<td>261.00±49.06</td>
</tr>
<tr>
<td>Histidine</td>
<td>41.67±25.45</td>
<td>229.14±409.46</td>
<td>318.33±440.93</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>80.50±21.93</td>
<td>78.57±17.09</td>
<td>67.83±26.82</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>232.67±36.34</td>
<td>211.14±16.44</td>
<td>225.33±14.11</td>
</tr>
<tr>
<td>Leucine</td>
<td>413.67±66.97</td>
<td>398.29±24.50</td>
<td>403.17±29.41</td>
</tr>
<tr>
<td>Lysine</td>
<td>1677.00±203.48</td>
<td>1545.30±303.21</td>
<td>1476.00±229.33</td>
</tr>
<tr>
<td>Methionine</td>
<td>147.00±25.88</td>
<td>171.00±27.42</td>
<td>156.83±20.34</td>
</tr>
<tr>
<td>Ornithine</td>
<td>126.83±15.05</td>
<td>109.43±26.91</td>
<td>134.50±26.37</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>338.17±22.10a</td>
<td>390.43±28.42b</td>
<td>307.50±29.97a</td>
</tr>
<tr>
<td>Proline</td>
<td>340.33±126.45</td>
<td>395.86±43.42</td>
<td>445.83±37.34</td>
</tr>
<tr>
<td>Serine</td>
<td>431.17±38.71a</td>
<td>528.29±64.04b</td>
<td>440.33±45.04a</td>
</tr>
<tr>
<td>Taurine</td>
<td>683.33±105.15</td>
<td>862.71±238.08</td>
<td>876.33±161.24</td>
</tr>
<tr>
<td>Threonine</td>
<td>514.00±63.00a,b</td>
<td>447.86±52.30a</td>
<td>563.50±54.72b</td>
</tr>
<tr>
<td></td>
<td>Alcohol Group</td>
<td>Pair-fed Group</td>
<td>Control Group</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>87.60±39.30</td>
<td>100.71±85.75</td>
<td>125.67±10.07</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>244.17±27.97a,b</td>
<td>281.71±49.41a</td>
<td>208.17±30.31b</td>
</tr>
<tr>
<td>Valine</td>
<td>514.33±70.85</td>
<td>491.29±72.69</td>
<td>501.83±59.74</td>
</tr>
<tr>
<td>Urea</td>
<td>5125.30±281.28a</td>
<td>2905.60±1104.00b</td>
<td>7032.00±908.46c</td>
</tr>
</tbody>
</table>

a,b,c Figures in the same row not sharing the same subscript are significantly different at least at the p<0.05 level.
Figure 4  Fetal Plasma Amino Acid Levels on Day 21 of Gestation (umoles/liter)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Alc</th>
<th>PF</th>
<th>Cont</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1305</td>
<td>1594</td>
<td>1194</td>
</tr>
<tr>
<td>ANBA</td>
<td>82</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>236</td>
<td>241</td>
<td>233</td>
</tr>
<tr>
<td>Asparagine</td>
<td>191</td>
<td>174</td>
<td>263</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>60</td>
<td>81</td>
<td>79</td>
</tr>
<tr>
<td>Cystine</td>
<td>25</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>475</td>
<td>558</td>
<td>552</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1492</td>
<td>1352</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>265</td>
<td>318</td>
<td>261</td>
</tr>
<tr>
<td>Histidine</td>
<td>42</td>
<td>230</td>
<td>318</td>
</tr>
</tbody>
</table>
Figure 4 continued

Hydroxyproline
AIC 81  
PF 79  
Cont 68  

Isoleucine
AIC 233  
PF 211  
Cont 225  

Leucine
AIC 414  
PF 398  
Cont 403  

Lysine
AIC 1677  
PF 1545  
Cont 1476  

Methionine
AIC 147  
PF 171  
Cont 157  

Ornithine
AIC 127  
PF 109  
Cont 135  

Phenylalanine
AIC 336  
PF 390  
Cont 308  

Proline
AIC 340  
PF 396  
Cont 446  

Serine
AIC 431  
PF 528  
Cont 440  

Taurine
AIC 683  
PF 863  
Cont 564  

57
### Figure 4 continued

<table>
<thead>
<tr>
<th></th>
<th>Threonine</th>
<th>Tryptophan</th>
<th>Tyrosine</th>
<th>Valine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AIC</strong></td>
<td>514</td>
<td>88</td>
<td>244</td>
<td>514</td>
</tr>
<tr>
<td><strong>PF</strong></td>
<td>448</td>
<td>101</td>
<td>282</td>
<td>491</td>
</tr>
<tr>
<td><strong>Cont</strong></td>
<td>564</td>
<td>127</td>
<td>208</td>
<td>502</td>
</tr>
</tbody>
</table>

(a) # corresponds to 39 umoles of amino acid per liter of plasma
Comparison of Maternal and Fetal Plasma Nutrient Levels

1) Folic Acid

Mean folic acid levels found in fetal rats at day 21 of gestation were considerably greater than levels found in the respective groups of dams. Folic acid levels in alcohol-treated and in control fetuses were approximately 7 times the levels found in their dams. Pair-fed fetuses had mean plasma folate levels 11 times greater than the levels in their dams.

2) Zinc

Mean plasma zinc levels were higher in fetuses than in dams at day 21 of gestation. Mean zinc levels were twice as high in control fetuses compared to control dams. Mean plasma zinc levels were 5 times higher in pair-fed and alcohol-treated fetuses than in their corresponding dams.

3) Magnesium

At day 21 of gestation, mean plasma magnesium levels were approximately 2–3 times higher in fetuses than in the dams.

4) Glucose

Mean plasma glucose concentrations in pair-fed and control fetuses were approximately 33% of that seen in pair-fed and control dams. Plasma glucose in alcohol-treated fetuses was 17% of that found in maternal plasma.

Comparison of Maternal and Fetal Plasma Amino Acid Levels

The mean amino acid levels of fetal plasma in all three treatment groups were generally higher than those in the corresponding dams. The only exception to this was cystine, for which maternal levels were slightly higher than fetal levels. Significant differences found in fetal amino
acid levels did not necessarily parallel differences in maternal amino acid patterns, this was true for the amino acids cystine, histidine, aspartic acid and tyrosine.

However, plasma threonine, serine and alanine concentrations showed a parallel between maternal and fetal groups. Mean maternal and fetal plasma threonine levels were lowest in the pair-fed group, but differed significantly only from the controls. Mean maternal and fetal plasma serine and alanine levels were significantly higher in pair-fed animals when compared to either the alcohol-treated or control groups.

Maternal plasma levels of proline were significantly lower in alcohol-treated compared to pair-fed or control dams. Fetal plasma proline levels showed the same trend as maternal levels, but the differences in fetal proline levels among the treatment groups were not significant.

Plasma phenylalanine levels in pair-fed fetuses were significantly higher when compared to both alcohol-treated and control fetuses. In maternal plasma phenylalanine was also highest in pair-fed animals, but the differences in maternal levels were not significant.

Lastly, mean plasma alpha-amino-\text{n}-butyric acid levels were significantly higher in alcohol-treated dams. Levels of ANBA were also higher in alcohol-treated fetuses, but due to the small number of fetal ANBA samples, a significant level of difference was not found.
DISCUSSION

The findings of this study are consistent with past work and indicate that prenatal alcohol exposure is deleterious to fetal development in the rat. No gross fetal malformations were observed, but as in numerous earlier reports (15,17-19,21-23,25), fetuses of alcohol-treated dams weighed less than fetuses of both pair-fed and control dams. This weight reduction occurred despite the pair-feeding design employed, indicating that the reduced weight of alcohol-treated fetuses is not due solely to reduced maternal food intake.

The National Academy of Sciences (72) has established nutrient requirements for growth and gestation of laboratory rats. These requirements are expressed in terms of nutrient concentration per kilogram of diet. During gestation, the requirements assume an average daily food intake of 20 grams and an average body weight of 300 grams in Sprague-Dawley rats. In the present study, alcohol-treated and pair-fed animals consumed an average of 11 grams of Purina Rat Chow/day throughout gestation. The average weight of alcohol-treated and pair-fed dams during gestation was 270 and 297 grams respectively.

Comparison of recommended nutrient intakes by the National Academy of Sciences (NAS) with the actual intake of nutrients by animals in the present study (determined by using Purina Rat Chow composition tables) revealed the following:

1. Alcohol-treated and pair-fed dams received an adequate amount of daily dietary folic acid throughout pregnancy. Furthermore, synthesis of folic acid by microflora can occur in the rat intestine. Thus, unless ethanol decreased intestinal absorption of folic acid, all dams in the
present study would be expected to have received an adequate amount of folic acid.

2. Throughout gestation alcohol-treated and pair-fed dams consumed an average of 0.33 mg. zinc/day and 23.0 mg. magnesium/day. These amounts exceed the established requirements of 0.24 mg. zinc/day and 8.0 mg. magnesium/day set by the NAS (72).

3. The National Academy of Sciences (72) has established a daily requirement for 13 amino acids to support optimum growth and gestation of rats. These amino acids are arginine, asparagine, glutamic acid, histidine, isoleucine, lysine, leucine, methionine, phenylalanine/tyrosine, proline, threonine, tryptophan and valine. The addition of arginine, asparagine, glutamic acid and proline to diets produces growth responses presumed to be due to the inability of the rat to synthesize sufficient quantities of these amino acids during rapid growth (72).

Comparison of the daily requirements established by the NAS (72) for these 13 amino acids, to levels consumed by rats in the present study, revealed that the average intake of arginine, histidine, isoleucine, leucine, lysine, threonine, tryptophan and valine by all three groups of rats exceeded requirements. However, the daily intake of methionine/cystine (85.8 mg.) and phenylalanine (111.7 mg.) by both alcohol-treated and pair-fed dams was below the requirement established by the NAS (120 mg. and 160 mg. respectively). When intake of methionine/cystine and phenylalanine were expressed relative to body weight, the amounts consumed by both alcohol-treated and pair-fed dams were still below the requirements.

4. The National Academy of Sciences (72) estimated that during gestation rats require 28 kcal./100 grams body weight (kcal. are expressed as gross energy available in the diet). In the present study, alcohol-
treated animals consumed an average of 27.3 kcal./100 grams body weight, and pair-fed animals consumed an average of 24.8 kcal./100 grams body weight throughout gestation.

Based on the NAS recommendations, the daily intake of folic acid, zinc, magnesium and most amino acids by alcohol-treated and pair-fed dams was adequate to support gestation. However, the lower food and calorie intake of alcohol-treated and pair-fed dams resulted in significantly less weight gain by these two groups compared to control dams.

Pair-feeding resulted in very similar prepregnancy rates of weight gain by alcohol-treated and pair-fed animals. However, throughout pregnancy alcohol-treated dams gained significantly less weight than pair-fed dams (p<0.005). Several other investigators have reported similar findings (16,21). This may indicate that ethanol at a level of 30% (v/v) in water reduces maternal nutrient utilization to a greater degree than 10 or 20% ethanol solutions. Maternal dehydration, produced by alcohol consumption, may also be increased by the higher concentration of alcohol. Alternatively, calories derived from alcohol may be less available than calories derived from cornstarch.

Results of this experiment indicate that weight gain prior to pregnancy is not correlated with fetal weight, while maternal weight gain during pregnancy is positively correlated with fetal body weight (r=0.6473, p<0.001). In humans, Metcoff (73) has estimated that total maternal weight gain during pregnancy contributes 6% to the variance in birth weight.

In this study no reduction in litter size of alcohol-treated dams occurred. This finding corroborates those of several other workers (17, 18,21-23,34). However, Singh and Snyder (74) did report a reduction in
litter size of rat dams fed a liquid diet starting on day 1 of pregnancy, with ethanol providing 36% of total calories. An explanation for this reduction in litter size may be provided by the findings of Rider (24). Rider compared the reproductive performance of rat dams fed 11% ethanol (in drinking water) for 13 days prior to and throughout gestation to the reproductive performance of dams started on an 11% ethanol solution on day 1 of pregnancy. The dams started on ethanol on day 1 of pregnancy had a reduced number of completed pregnancies and smaller litters than the dams fed ethanol for the longer period of time. Also, Abel and Greizerstein (21) noted that rat dams intubated daily with 6 grams ethanol/kg. body weight, from day 5 of gestation, showed a higher rate of fetal resorption than dams intubated with 6 grams ethanol/kg. body weight commencing on day 1 of gestation. These and the present results suggest that short term alcohol consumption reduces the reproductive potential of rat dams, whereas the dams can adapt somewhat to chronic alcohol consumption. However, fetal growth is compromised by both short term and chronic maternal alcohol consumption.

In the present study, no increase in placental weight was found in alcohol-treated dams compared to controls. Both Jones et al. (30) and Weiner et al. (27) have reported higher placental weights in alcohol-treated dams compared to pair-fed and control dams. Jones et al. (30) suggested that increased placental weight may represent a compensation for reduced blood flow to the placenta. However, in the present study evidence of hypoglycemia was found in alcohol-treated fetuses, indicating that compensatory placental hypertrophy is not adequate to maintain normal fetal blood glucose levels. No correlation was found between placental weight and fetal body weight.
Our results show that maternal duodenal conjugase activity (necessary for the hydrolysis of folate polyglutamates prior to absorption), is not impaired by alcohol consumption. Alcohol-treated dams would therefore have received an adequate supply of folic acid both from dietary sources and from intestinal microflora. However, from the present results it cannot be concluded that the dietary treatments had no effect upon maternal plasma folate. Although significant differences were not found in maternal plasma folate levels, the means for alcohol-treated and pair-fed groups were one half or less of the mean of the control group. In addition, the samples sizes were small, and the standard deviation of the alcohol-treated group was large. Determination of folate levels in a larger sample may reveal differences among the three treatment groups.

Lin (75) reported that plasma folate levels were significantly lower in alcohol-treated and pair-fed dams compared to control dams at day 21 of gestation. However, in agreement with the present results, she found no differences in plasma folate levels between alcohol-treated and pair-fed dams.

Also similar to our results, Lin (75) found no significant differences in plasma folate among alcohol-treated, pair-fed and control fetuses at day 21 of gestation. These and the present results indicate that ethanol does not seem to inhibit placental transport of folate.

However, we cannot conclude from the present study that ethanol has no effect upon maternal or fetal folate status. Ethanol may affect erythrocyte, bone marrow or liver folate levels. Frank and Baker (46) reported that alcohol consumption by male rats produced a decrease in blood total folate levels when compared to pair-fed animals. Switzer
et al. (76) reported that ethanol significantly decreased mean erythrocyte folate levels in dogs, but had no effect upon mean plasma folate levels, when compared to pair-fed animals. Carney and Sheffield (77) reported that mean serum folate levels in alcoholics did not differ from those in nonalcoholic subjects. However, there is also some evidence that ethanol directly reduces the formation of normoblastic bone marrow, and decreases hepatic retention of folic acid (78). Thus, the effects of ethanol on folate status may vary depending upon whether blood, plasma, bone marrow or liver folate is measured.

No significant differences were found in plasma zinc levels among the three groups of dams. This is in contrast to several reports of abnormally reduced serum zinc concentration (41) or increased renal clearance of zinc (42) found in human alcoholics. Our results are also at variance with those of Wang and Pierson (44), who reported decreased plasma zinc levels in male rats given ethanol for 9 weeks, compared to controls. However, similar to the present study, Suh and Firek (79) found no significant differences in plasma zinc levels between pregnant rats given a 24% ethanol solution and a pair-fed group. Discrepancies among studies may be due to differences in the levels of dietary zinc consumed. In the present study, alcohol-treated dams consumed daily amounts of zinc in excess of requirements for pregnant animals. Daily consumption of zinc was not reported in other studies.

In the present study, plasma zinc levels did not differ significantly between alcohol-treated and pair-fed fetuses. Hurley and Mutch (80) reported that mean plasma zinc levels in normal rat fetuses at day 21 of gestation was approximately 440 µg./100 ml. This value is comparable to the mean levels of plasma zinc found in the present study in alcohol-
treated and pair-fed fetuses (492 and 576 ug./100 ml respectively). These results indicate that maternal alcohol consumption did not reduce the availability of zinc to the alcohol-treated fetuses. However, Hurley and Mutch (80) fed rat dams a zinc deficient diet only from days 6 to 14 of gestation and compared pregnancy outcome of these animals to that of controls fed a diet containing adequate zinc. They found that zinc deficiency produced a high rate of stillbirths and neonatal abnormalities, and a reduction in the weight of day 16 and day 21 fetuses. Despite these findings, no significant differences in concentration of plasma or tissue zinc occurred at any age between control and zinc-deficient fetuses. Thus it seems that fetal plasma zinc concentration is not a sensitive index of zinc status. An effect of ethanol upon zinc status may only be evident by studying some other body zinc pool, such as zinc-dependent metalloenzymes.

The present study indicates that ethanol does not reduce maternal or fetal plasma magnesium levels. Magnesium levels were found to be significantly higher in alcohol-treated dams when compared to both pair-fed and control dams. The high plasma magnesium levels in the alcohol-treated group were unexpected, because alcohol consumption has been reported to cause increased urinary loss of magnesium, and to be associated with magnesium deficiency (43). However, Suh and Firek (79) reported that when female rats were given a 24% ethanol solution for 2-8 weeks prior to gestation and compared to pair-fed rats, no differences were found in plasma magnesium levels between the two groups. The results of the present study are in agreement with those of Abel and Greizerstein (21). They reported that intubation of pregnant rats with 6 grams ethanol (30% w/v)/kg. body weight, from
days 5 to 19 of gestation, produced significantly higher mean plasma magnesium levels when compared to pair-fed dams (approximately 2.3 mg./100 ml. and 1.97 mg./100 ml. respectively), at day 20 of gestation. These and the present findings may be due to increased absorption of magnesium or to increased mobilization of magnesium from maternal bone and muscle. In humans, alcohol ingestion is known to cause degeneration of muscle tissue (65,66).

Maternal alcohol consumption had no effect upon fetal plasma magnesium concentration. Despite an elevation in the plasma magnesium levels of alcohol-treated dams, increased plasma magnesium levels were not found in alcohol-treated fetuses. Abel and Greizerstein (21) also reported no differences in magnesium concentration of amniotic fluid, placentae, or whole fetuses between alcohol-treated and pair-fed animals, despite significantly higher plasma magnesium levels in alcohol-treated dams compared to pair-fed dams. These results may be an indication of a saturation mechanism of placental transport of magnesium. Alternatively, some of the magnesium in the plasma of alcohol-treated dams may be present in a form which is unavailable for placental transport (ie. complexed with protein).

It was anticipated that alcohol consumption might cause hypoglycemia due to inhibition of hepatic gluconeogenesis (47,48). Alcohol-treated dams consumed fewer calories (kcal./gram body weight) than did control dams. A low intake of calories would be expected to cause mobilization of maternal body fat and release of muscle alanine to provide substrates for gluconeogenesis. However, Scholz et al. (81) reported that perfusion of rat livers with alanine and oleate (a condition designed to mimic hypoglycemia) caused stimulation of gluconeogenesis, while addition of
ethanol to the perfusate diminished the stimulation of gluconeogenesis. In the present study, no significant differences were seen in maternal plasma glucose among the three treatment groups. Thus, inhibition of gluconeogenesis does not seem to be a factor in pregnant dams consuming alcohol.

In agreement with our results, Snyder and Singh (49) found no significant differences in maternal blood glucose levels among alcohol-treated, pair-fed and control dams, when blood samples were taken at term. Also, in a study reported by Wallgren et al. (82), no differences in plasma glucose levels were detected between a group of male rats given a 3-9% ethanol solution and a pair-fed group.

In contrast to glucose levels in maternal plasma, the mean fetal plasma glucose levels in the present study were significantly lower in alcohol-treated compared to both pair-fed and control groups. Snyder and Singh (49) also found significantly reduced blood glucose levels in the newborn pups of their alcohol-treated dams when compared to either pair-fed or control pups.

The reason that alcohol-treated fetuses should display evidence of hypoglycemia while their dams did not, is not readily apparent. Glucose is transported across the placenta via facilitated diffusion (83). Jones et al. (28) found that alcohol consumption by rat dams did not diminish fetal uptake of a non-metabolizable glucose analog (methyl-(alpha-D(U-14C)gluco)pyranoside). Thus reduction of glucose transport across the placenta by ethanol seems unlikely.

In fetal rats alcohol dehydrogenase activity has not been detected until approximately day 18 of gestation (84). Following day 18 it is not known what effect ethanol oxidation may have on fetal gluconeogenesis.
However, there is evidence that metabolic response to ethanol varies with age. Hollstedt et al. (85) found that ethanol injected intraperitoneally (27.1 mM/kg. body weight), produced hypoglycemia in rats weighing 25 grams, but not in 50, 100, 200 or 250 gram rats.

The effect of hypoglycemia beginning on prenatal day 18 upon fetal growth is unknown. However, because fetal growth is very rapid during the last few days of gestation, hypoglycemia during this time may result in significant growth retardation. Further studies examining fetal growth and plasma glucose levels prior to day 18 of gestation may clarify this finding.

Are alcohol derived calories available to the fetus? In the present study, alcohol-treated dams consumed approximately 33% of daily calories as ethanol throughout gestation. In humans, it has been estimated that approximately 75% of ethanol taken up by the liver is released into the circulation as acetate (31). Acetate can be utilized by maternal tissues as an energy source. However, it is unknown if acetate crosses the placenta or can be utilized by fetal tissues.

Plasma osmolality was increased significantly in alcohol-treated dams compared to both pair-fed and control dams. Similar findings were reported by Jones et al. (28). Redetzki et al. (86) found a direct relationship between blood alcohol concentration and serum osmolality in 50 alcoholic patients. They reported that factors which contributed to the increase in osmolality included increased levels of serum lactic acid, acetate and alcohol congeners.

In the present study, no differences in plasma lactate were found among the three fetal groups. However, because lactate was measured in plasma which had not been deproteinized immediately, the values obtained
may not be reliable.

The effect of maternal dehydration upon fetal growth is unknown. However, Jones et al. (30) reported that alcohol-induced maternal dehydration in rats was associated with reduced blood flow to placentae. This reduction in blood flow may reduce the supply of nutrients available to fetuses of alcohol-treated dams.

Only seven of the maternal plasma amino acids and six of the fetal plasma amino acids showed significant differences among the three treatment groups. In the dams, reduced plasma threonine, cystine and histidine levels were apparently a result of reduced food intake, as the levels of these amino acids were similar in alcohol-treated and pair-fed dams, but significantly lower than those of control dams. Reduction in these plasma amino acids in response to protein-calorie malnutrition has been found in other studies. Worthington et al. (87) found a 68% reduction in serum cystine, and a 32% reduction in serum threonine in adult monkeys after 4 weeks of severe protein-calorie malnutrition.

In addition, protein-calorie malnutrition results in an increase in muscle release of alanine (88). In the present study, pair-fed dams had significantly greater plasma alanine and serine levels than both alcohol-treated and control dams. An increase in serum serine (6%) and alanine (44%) were also found in the study of Worthington et al. (87) in response to protein-calorie malnutrition.

A decrease in the ratio of plasma essential to nonessential amino acids has also been found in protein-calorie malnutrition. Comparison of this ratio in the maternal plasma of the three treatment groups, showed that indeed it was less in pair-fed (0.75) compared to alcohol-
treated (0.92) and control (1.38) animals. Thus, it is possible that pair-fed dams may have been suffering from mild protein-calorie malnutrition. However, this had no significant effect on the body weight of pair-fed fetuses when compared to control fetuses.

Significantly elevated levels of alanine, phenylalanine, tyrosine and serine were found in pair-fed fetuses compared to controls. The elevation of fetal plasma serine and alanine are a reflection of similar elevations of these amino acids in the plasma of pair-fed dams.

Alcohol consumption did produce changes in a few maternal and fetal plasma amino acids in alcohol-treated animals when compared to pair-fed animals. These amino acids included maternal alpha-amino-n-butyric acid and proline, and fetal aspartic acid and alpha-amino-n-butyric acid.

Plasma levels of proline were significantly depressed in alcohol-treated dams. Mean proline levels were also depressed in alcohol-treated fetuses, although due to the large standard deviation value, differences in fetal plasma proline were not significant. A deficiency of proline may affect fetal skeletal development. Proline is a precursor in the formation of collagen, and has been shown to be an essential amino acid for rats during rapid growth (72). In addition, studies have reported retardation of skeletal development in the offspring of alcohol-treated dams (15,22,25). Leichter and Lee (22) measured arm length, leg length, pelvic width, pelvic length, skull width and skull length in the pups of alcohol-treated, pair-fed and control dams at 30, 37, 44 and 51 days postconception. For every parameter, at every measurement period, skeletal maturity of the alcohol-treated pups was significantly less than that of pair-fed and control pups.

Mean plasma aspartic acid levels were significantly depressed in
alcohol-treated fetuses but not alcohol-treated dams. This may indicate that alcohol reduces placental transport of some amino acids. In a study of normal placental function, Eaton and Yudilevich (90) perfused isolated guinea pig placentae with tritiated amino acids and measured placental uptake. They found that uptake of aspartic acid was minimal (approximately 5% compared to 55% for isoleucine). Other amino acids which showed small percentage uptake by the placentae were glutamic acid (approximately 1%) and taurine (approximately 13%). In the present study, plasma glutamic acid and taurine levels were lower in alcohol-treated fetuses compared to pair-fed and control fetuses, although the differences were not statistically significant.

Reduction of the supply of acidic amino acids to alcohol-treated fetuses may be a result of reduced maternal blood flow to placentae. Reduced supply of acidic amino acids may have relevance to fetal development. Studies have suggested that glutamic acid is an essential amino acid for rapidly growing rats (72). Aspartic acid is an essential precursor in pyrimidine and purine synthesis.

Holt et al. (89) reported that kwashiokor in young children was accompanied by a decrease in plasma alpha-amino-n-butyric acid, and an increase in plasma proline. In contrast, alcohol-treated dams in our study had significantly elevated plasma alpha-amino-n-butyric acid and depressed plasma proline levels. A correlation also existed between fetal plasma alpha-amino-n-butyric acid (ANBA) levels and fetal body weight. These results indicate that alcohol has a unique effect upon plasma amino acids irrespective of reduced food intake.

Preliminary findings by Shaw and coworkers (91,92) indicate that the ratio of the concentration of plasma alpha-amino-n-butyric acid to
leucine (A/L) may be used as a test for alcoholism. In humans, they found that the ratio of these two amino acids was elevated for several weeks after cessation of chronic drinking, but was not affected by acute alcohol ingestion. The elevated ratio was observed in both well-nourished and poorly nourished alcoholics. Baboons fed alcohol along with a protein deficient diet developed an abnormal A/L ratio when alcohol was given, but not when alcohol was omitted (91). Furthermore, it was found that in patients with acute and chronic hepatitis, the ratio was not significantly different from controls (91).

Thus in the present study, elevated plasma levels of ANBA in alcohol-treated rats may reflect chronic alcohol consumption rather than the occurrence of liver damage. Evidence that liver damage did not occur in the dams is supported by the fact that changes in plasma protein and amino acids generally associated with alcoholic liver disease did not occur. Low levels of serum albumin have been associated with liver cirrhosis (31). In the present study, no significant differences were found in maternal plasma albumin among the three treatment groups. Also, in patients with chronic active liver disease, an abnormal elevation of serum phenylalanine and tyrosine and a decrease in the ratio of serum branched-chain amino acids to aromatic amino acids were found (93). These changes were not seen in the alcohol-treated animals of the present study.
The present study has shown that ethanol consumption by rats for 5 weeks prior to and throughout gestation results in reduced fetal weight. This reduction was independent of total maternal food intake.

Fetal growth retardation was not associated with any abnormal findings in maternal plasma folic acid, zinc, glucose or albumin; or in fetal plasma folic acid, zinc, or magnesium. However, in the future, observation of parameters other than plasma nutrient levels may show that alcohol consumption does affect the status of these nutrients in either rat dams or fetuses. For example, alcohol consumption may reduce incorporation of folate into erythrocytes, or may reduce hepatic zinc and folate retention.

Alcohol consumption was found to increase maternal plasma osmolality. The effect of this upon fetal growth is unknown.

Increase in maternal plasma magnesium was found as a result of alcohol consumption. This may be a result of muscle tissue mobilization.

Plasma levels of proline were decreased in alcohol-treated dams, which may compromise fetal collagen formation. Levels of aspartic acid were reduced in alcohol-treated fetuses, which may have an effect on pyrimidine and purine synthesis and ultimately the formation of DNA and RNA and cell multiplication.

A positive correlation was found between maternal plasma proline levels and fetal body weight, and a negative correlation between maternal plasma alpha-amino-n-butyric acid levels and fetal body weight. A negative correlation was also observed between fetal plasma ANBA and fetal body weight. These plasma amino acids may prove to be useful indicators of maternal alcoholism during pregnancy and of the risk of
fetal alcohol syndrome occurring in the developing fetus. However, the mechanisms whereby alcohol affects amino acid metabolism remain to be elucidated.

Lastly, results suggest that reduced plasma glucose concentration in alcohol-treated fetuses is due to inhibition of hepatic gluconeogenesis. Decreased fetal body weight produced by maternal alcohol consumption was positively correlated with fetal plasma glucose levels. Thus, fetal growth retardation in alcohol-treated rats may result from hypoglycemia.
REFERENCES


