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Effect of Intrauterine Ethanol Exposure on Fetal Lung Growth

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ABSTRACT. Lung weight, DNA, RNA, protein, and total body weight were analyzed in fetuses from 14 pregnant Sprague-Dawley rats fed a nutritionally complete liquid diet containing v/v 6% ethanol (ethanol group). Each ethanol rat was matched with an isocalorically pair-fed animal (control group) who received the same liquid diet with carbohydrate substituted for ethanol. The rats were killed on day 20 of gestation. The mean maternal blood ethanol concentration at 0700 h on day 20 was 170 ± 22 (SE) mg/dl. Compared to controls, ethanol fetuses had reduced body weight (21%), lung dry weight (39%), lung wet weight/body weight ratio (10%), DNA (21%), RNA (25%), protein (28%), and protein/DNA ratio (8%) ($p < 0.05$). The results indicate that prenatal ethanol exposure inhibits cellular growth in the fetal lung, resulting in hypoplastic lungs which have fewer and smaller cells. The effect on the lung appears to be greater than on the body as a whole. These hypoplastic lungs may be predisposed to the development of pulmonary disease and may explain observations of more frequent and severe lower respiratory infections in children with prenatal ethanol exposure. (*Pediatr Res* 19: 12–14, 1985)

Abbreviation

FAS, fetal alcohol syndrome

Prenatal ethanol exposure results in a spectrum of growth disturbances known as the FAS and includes intrauterine and postnatal growth retardation, facial dysmorphogenesis, and CNS dysfunction (30). Hypoplasia and malformations of different organs are described both clinically and experimentally in the FAS (8, 15, 23, 30).

Children with the FAS have an increased frequency and severity of lower respiratory tract infections, which have been previously attributed to immunologic deficiencies induced prenatally by ethanol (19). In addition, decreased production of lung surfactant, decreased activity of pulmonary alveolar macrophages and cilia, and impaired migration of polymorphonuclear leukocytes to the lung occur with ethanol exposure in the adult (2, 13, 14, 31).

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Ethanol may also affect the growth and development of the lung. Since lung growth is characterized by the formation of all pulmonary airways and air spaces during gestation (18, 22), intrauterine ethanol exposure may alter prenatal lung growth and predispose the lung to the future development of disease. Therefore, the effect of chronic prenatal ethanol ingestion on fetal lung growth was studied in rats.

MATERIALS AND METHODS

Eight mature female Sprague-Dawley rats (ethanol group) were acclimated over 10 days to a nutritionally complete liquid diet, modified for pregnancy with additional vitamins and minerals (Lieber-DeCarli; BioServ Inc, Frenchtown, NJ). The ethanol diet contained v/v 2% ethanol for 3 days, 4% for 3 additional days, and 6% thereafter. Each ethanol rat was matched with an isocalorically pair-fed animal (control group) who received the same liquid diet with carbohydrate substituted for ethanol. Both liquid diets have a caloric density of 1 cal/ml with 18 and 35% of the calories provided by protein and fat, respectively. Ethanol provides approximately 35% of the calories in the 6% ethanol diet.

During the mating period the liquid diets were discontinued and the rats were fed Purina Chow (Ralston Purina no. 5001, St. Louis, MO) *ad libitum*. When pregnant, as ascertained by vaginal smear, they were returned to their respective liquid diets.

The day the vaginal plug was first observed was designated as day 0 of pregnancy. At 0700h on pregnancy day 20, blood ethanol concentrations were determined by the alcohol dehydrogenase method (Ethyl Alcohol Stat-Pak; Calbiochem-Behring, La Jolla, CA) (5). The rats in both groups were then weighed and killed by cervical dislocation. To eliminate any possible diurnal variations in cellular response, all animals were killed between 9.00 and 11.00 h (7).

Gross abnormalities and number of fetal resorptions were noted. The fetuses were removed, weighed, and immediately placed on ice. Their lungs were dissected, weighed, and immediately frozen for biochemical analyses. Since individual fetal lungs were too small for nucleic acid analysis, three to seven lungs from each litter were pooled and homogenized. Lung tissue nucleic acids were extracted by a modified Schmidt-Thannhauser procedure (26). DNA and RNA were determined by UV absorption at 268 and 260 nm, respectively. Lung tissue protein was determined by the Lowry method (24). DNA, RNA, and protein standards were obtained from the Sigma Chemical Company (St. Louis, MO).

Since all fetal lungs from these eight pairs of rats were used for biochemical analyses, the effect of ethanol on the fetal lung dry weight/wet weight ratio was determined in fetuses from an additional six pregnant Sprague-Dawley rats who were fed the 6% ethanol diet. Isocalorically pair-fed matched pregnant controls received the carbohydrate-substituted liquid diet. Maternal

blood ethanol levels were obtained at 07.00h, and the rats were killed by a combination of pentobarbital and ether. The fetuses and their lungs were individually weighed. Lungs from each of these fetal litters were pooled and dried to constant weight at 60° C.

Since there was no difference in ethanol intake between the two groups of ethanol-fed mothers, their data for weight gain, blood ethanol levels, and litter size were combined. Similarly, fetal data for body and lung weights were combined.

A mean value for each measurement was obtained per litter. Statistical significance of the data was assessed by the paired t test (3) in which each ethanol-treated litter was matched with its isocalorically pair-fed control. Results are expressed as means ± SE and are considered significant for $p < 0.05$.

RESULTS

There was no difference in maternal weight gain between the ethanol (83 ± 8 g, $n = 14$) and control (79 ± 5 g, $n = 14$) groups. Litter size was similar, with 11.6 ± 0.8 ethanol fetuses/litter ($n = 14$) and 12.1 ± 0.7 control fetuses/litter ($n = 14$). Maternal daily ethanol intake was 11.6 ± 0.3 g/kg ($n = 14$), and the maternal blood ethanol concentration was 170 ± 22 mg/dl ($n = 9$).

Three fetal reabsorptions and one stillbirth occurred in two ethanol dams, and one control dam had a reabsorption. Gross abnormalities were absent in the fetuses. There was no gross evidence of infection in the maternal and fetal lungs, and all lungs at the termination of the study were uniformly inflated and homogeneously pink in color.

Compared to controls, the ethanol fetuses had significant reductions in body weight (21%) (Table 1). Not only were lung wet and dry weights significantly diminished by 29 and 39%, respectively, but the lung wet weight/body weight ratio was also significantly lower by 10% (Table 1), indicating the presence of smaller lungs. Significant decreases in DNA (21%), RNA (25%), protein (28%), and the protein/DNA ratio (8%) were also present in the ethanol fetuses as compared to the control fetuses (Fig. 1 and 2). The RNA/DNA ratio remained unchanged. This indicates inhibition of cellular growth as measured by DNA content, which reflects cell number, and by the protein/DNA ratio, which reflects cell size (18).

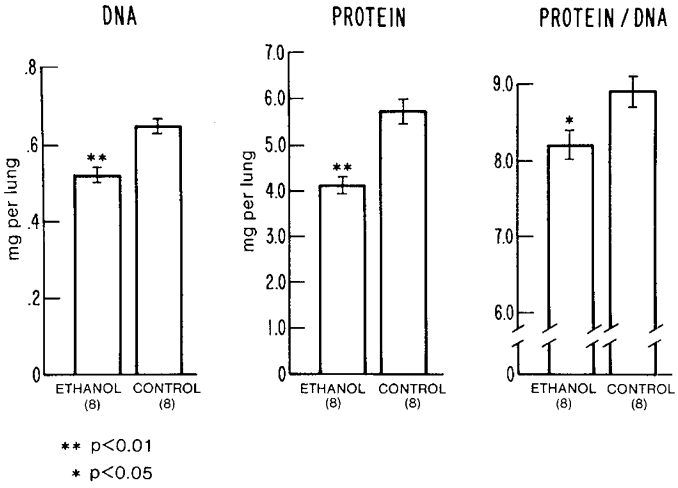

Fig. 1. Comparison of cell number (DNA content) and cell size (protein/DNA ratio) in ethanol-exposed and control fetal rat lungs. Means ± SE are shown. Numbers in parentheses represent number of litters.

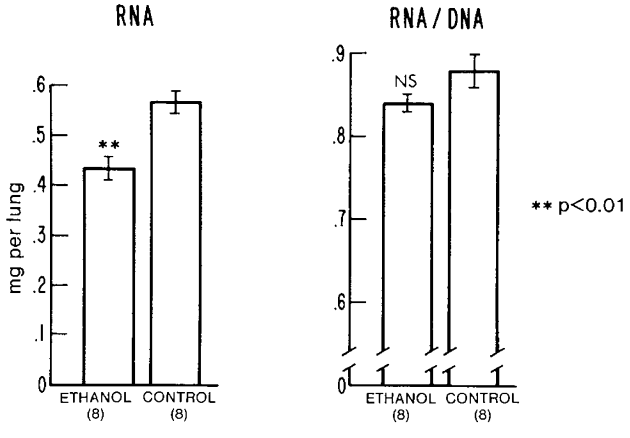

Fig. 2. Comparison of RNA content and RNA/DNA ratio in ethanol-exposed and control fetal rat lungs. Means ± SE are shown. Numbers in parentheses represent number of litters.

DISCUSSION

The results of these studies demonstrate that chronic exposure to high doses of ethanol during gestation alters the growth and development of the fetal lung. Diminished lung cell number and size characterize these hypoplastic lungs. Intrauterine ethanol exposure inhibits DNA and protein synthesis in the fetal lung, a finding consistent with observations of inhibition of DNA and protein synthesis in other organs (11, 15–16).

Ethanol exposure in this model as well as in the human occurs during the early stages of lung development when the branching pattern of the pulmonary airways is forming (18). Since prenatal ethanol exposure inhibits cellular growth in the lung, it is possible that ethanol alters the branching pattern, resulting in diminished numbers of airways and subsequently diminished numbers of alveoli (18). However, morphologic studies are necessary to delineate further the inhibitory role of ethanol on fetal lung growth.

Table 1. *Fetal body and lung weights (means ± SE.)*

	Ethanol group	Control group
Body wt/fetus (g)	3.04 ± 0.18** (14)	3.87 ± 0.20 (14)
Lung wet wt/fetus (mg)	69.2 ± 5.9** (14)	97.8 ± 5.5 (14)
Lung wet wt/body wt ($\times 10^{-2}$)	2.28 ± 0.09* (14)	2.54 ± 0.09 (14)
Lung dry wt/fetus (mg)	7.0 ± 0.4** (6)	11.4 ± 0.4 (6)
Lung dry wt/wet wt ($\times 10^{-2}$)	13.2 ± 0.3 (6)	13.0 ± 0.6 (6)

Numbers in parentheses represent number of litters.
* $p < 0.05$.
** $p < 0.01$.

The model used in the present study results in maternal blood ethanol concentrations which are comparable to human levels during chronic heavy drinking. Unlike other models of chronic ethanol consumption during pregnancy (1, 15), maternal weight gain and litter size are not altered and therefore do not contribute to the observed fetal differences. With the use of pair-fed controls, our results are independent of maternal nutrient intake. Yet, intrauterine growth retardation does occur.

The etiology for hypoplastic lung growth with chronic prenatal ethanol exposure is unknown. Several mechanisms might be implicated and include direct toxicity of ethanol or its metabolites (4, 11, 16, 28), impaired placental transport of nutrients (10, 27) or oxygen (20, 25, 27), hypoxia-induced pulmonary vasoconstriction potentiated by ethanol (9, 12, 17), alterations in activities of pulmonary chemical mediators (6, 18, 21, 29), and diminished surfactant synthesis (31). Regardless of the mechanism of action, the reduction in lung size relative to body weight indicates that the lung is particularly susceptible to intrauterine ethanol exposure.

Pulmonary disorders have not been previously considered to be a manifestation of the FAS. However, the present experiments indicate that the lung is a target organ for ethanol-induced

alterations in cellular and organ growth. Additional studies are necessary to evaluate the etiology and mechanisms of ethanol-induced prenatal pulmonary hypoplasia. The present investigations have particular clinical importance in the pediatric population who may be exposed to environmental toxins before birth and may have significance in the prevention of pulmonary disease in this age group.

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