

EFFECTS OF ALCOHOL INGESTION IN THE PREGNANT RAT ON DAILY FOOD INTAKE, OFFSPRING GROWTH AND METABOLIC PARAMETERS

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Abstract—1. Daily food intake, fluid consumption, offspring growth and metabolic parameters were studied in rats receiving ethanol in the drinking water before, during and after gestation.

2. Ethanol treatment always reduced daily food, liquid and caloric intake in the rat, except during gestation when total daily caloric intake was greater in rats receiving ethanol than in control animals. The increase in both food and liquid intake during lactation in controls was also observed in alcohol-deprived mothers but was significantly reduced in mothers maintained under alcohol treatment.

3. Offspring from alcohol-treated mothers were retarded in body weight and size compared with controls, the differences becoming greater as the suckling period advanced.

4. The 15 day-old pups from alcohol-treated mothers had reduced glucose and augmented beta-hydroxybutyrate levels in blood, and markedly reduced liver glycogen concentrations, indicating their acutely denutrited state.

5. In pups from mothers that received alcohol until the 21st day of gestation, body weight and size were normalized at the 15th day but skeletal maturation and liver glycogen concentration were reduced and blood acetoacetate and beta-hydroxybutyrate were augmented as compared with values in pups from control mothers. If these parameters are interrelated, metabolic changes may be used for early diagnosis of fetal alcohol syndrome.

INTRODUCTION

It is well known that offspring of women who consume large amounts of alcohol during pregnancy are characterized by a pattern of congenital abnormalities called the fetal alcohol syndrome (Jones & Smith, 1973). Pre- and post-natal growth retardation is a characteristic feature of the fetal alcohol syndrome in both humans and laboratory animals (Jones & Smith, 1973; Streissguth *et al.*, 1980; Leichter & Lee, 1979; Herrera & Llobera, 1981). Malnutrition in alcoholic mothers is not considered an important issue in the growth retardation of their offspring (Abel & Dintcheff, 1978; Lee & Leichter, 1980), but placental transport of nutrients is impaired (Lin, 1981; Henderson *et al.*, 1981), producing fetal malnutrition even though nutrients within the maternal circulation are adequate. During both pregnancy and lactation in the rat, the mother voluntarily increases her daily food intake (Kumaresan & Turner, 1968; Knopp *et al.*, 1975; Champigny *et al.*, 1980) to compensate for the continuous draining of nutrients by the fetus or by the milk formation. The present study was performed to determine the effect of chronic alcohol intake in the rat mother on caloric intake during gestation and post-partum when fed *ad libitum* and also the effect of alcohol withdrawal from the 21st day of gestation on

dietary parameters in the mother. The study was extended to determine growth and metabolic changes in 15 day-old suckling pups of ethanol treated mothers.

MATERIALS AND METHODS

Female Sprague-Dawley rats of 169 ± 5 g body wt from our own colony were maintained under automatically controlled temperature ($25 \pm 1^\circ\text{C}$) and 12 hr light-dark cycles (9:00 to 21:00 hr). They were divided into three groups: (1) Alcohol-treated, receiving 10% ethanol (w/v) in drinking water for one week, 15% ethanol during the second week, 25% ethanol during the third week and 30% ethanol from the fourth week. At the end of the fourth week, they were mated with normal males and they received 30% ethanol in drinking water during gestation and two weeks of lactation, after which they were sacrificed (at the 15th day post-partum). (2) Alcohol-deprived with ethanol withdrawn at the 21st day of gestation. These rats received the same ethanol treatment as the alcohol-treated (group 1) until the 21st day of gestation after which they received tapwater. (3) Controls, sex and age matched and handled in the same way as the other two groups but always given tapwater. All rats were housed 6 per cage until mating time, after which they were kept 2 per cage until parturition, when each mother was kept in a separate cage with her respective pups. All had free access to both purina chow diet and drinking solution throughout the experiments. Food and drinking solution consumed and body weights were measured daily. Parturition time and litter sizes were noted and offspring number was reduced to 8 per mother from the time of parturition. Offspring were fed *ad libitum* by their respective mothers and were sacrificed by decapitation between 9:30 and 11:30 am on the 15th post-natal

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day. Blood was collected from the neck into heparinized containers and aliquots of whole blood were immediately deproteinized with $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$ (Somogyi, 1945). Supernatants were stored frozen for subsequent analysis of glucose (Huggett & Nixon, 1957), acetoacetate (Mellamby & Williamson, 1974), and beta-hydroxybutyrate (Williamson & Mellamby, 1974) by enzymatic methods. Livers were excised immediately after sacrifice and placed in liquid N_2 . Liver glycogen was purified with ethanol after alkali digestion (Good *et al.*, 1933) and hydrolyzed with 5 N H_2SO_4 at 100°C for 2 hr after which it was neutralized for the evaluation of glucose (Huggett & Nixon, 1957). Skeletal maturity was estimated in 8 and 15 day-old offspring maintained under light ether anesthesia according to the procedure of Hughes & Tanner (1970). Animals examined for skeletal maturity were different than those used for metabolic parameter estimations, but they were maintained under the same conditions. Results are expressed as means \pm SE of

the means, and statistical comparisons were done by the Student's *t*-test.

RESULTS

In Figs 1a and b, the values of daily food and fluid intake are summarized for the most characteristic days in the three groups of animals studied. In control rats, during the first three weeks of experiments (pregestation period) there was almost no change in food intake, whereas water intake increased progressively. In controls during gestation, there was a gradual, marked rise in both parameters lasting until parturition when there was a temporary decrease in food intake and a further enhancement of water intake. Lactation produced a greater increase in both food

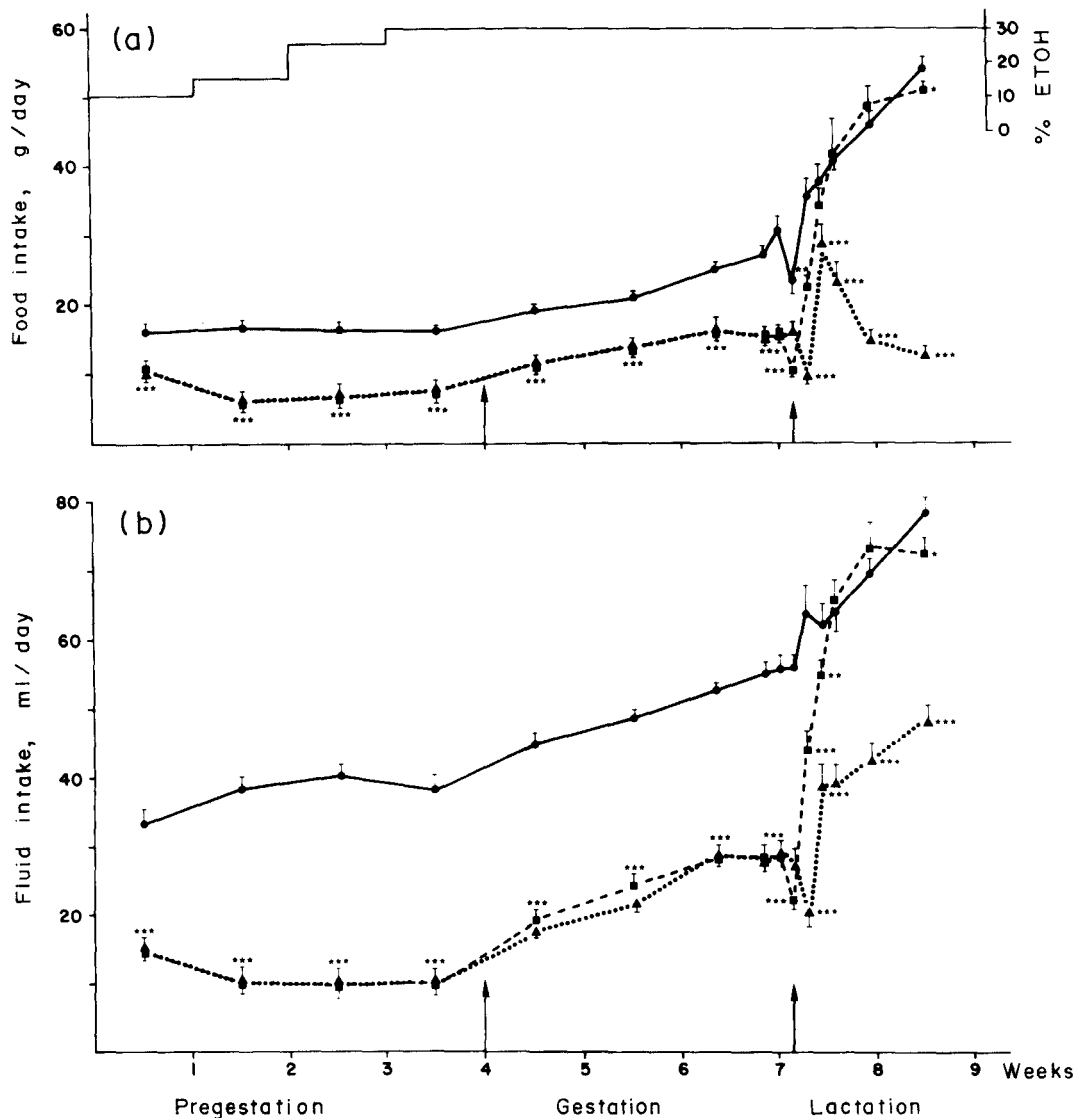


Fig. 1. Daily food (a) and fluid (b) intake in the rat under alcohol treatment. Rats received ethanol in drinking water at the concentrations shown in the top of the figure throughout the whole experiment ($\Delta \cdots \Delta$) or until day 21st of pregnancy ($\square \cdots \square$). Age and sex matched controls ($\bullet \cdots \bullet$) received water. Arrows corresponds to either onset of gestation or parturition time. Means \pm SEM. *P* values vs controls are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. *n* = 20–40/group.

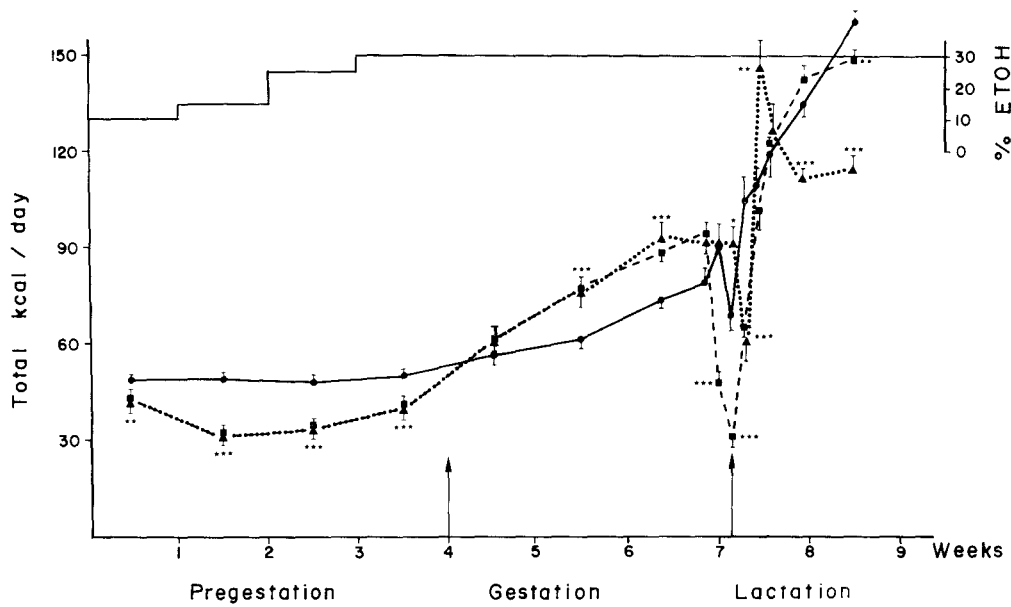


Fig. 2. Total daily caloric intake in the rat under alcohol treatment. Specifications as in Fig. 1.

and water intake in controls (Figs 1a and b) which was maintained until the 15th day post-partum. In rats receiving ethanol in the drinking water, both daily food and fluid consumption were greatly reduced (Figs 1a and b) as compared with control values, but alcohol ingestion did not impair the increase in these parameters produced with gestation. The enhancement of both food and fluid intake during gestation in ethanol mothers was even greater than in controls (Figs 1a and b). During parturition there was also a temporary reduction in both food and fluid intake in the alcoholic mothers but with lactation, an initial rise in food intake only lasted

until the 4th day after which it decreased markedly. Fluid intake in the alcohol-treated mothers increased progressively with lactation but these values were always lower than in controls (Fig. 1b). When alcohol-treated mothers were deprived of alcohol after the 21st day of gestation, they rapidly regained both daily food and fluid intake values to the levels in controls during lactation (Figs 1a and b).

Total daily caloric intake (Fig. 2) was significantly reduced during the pregestational period in ethanol rats compared with their controls. These differences quickly disappeared during gestation and was even reversed from the second week of pregnancy until

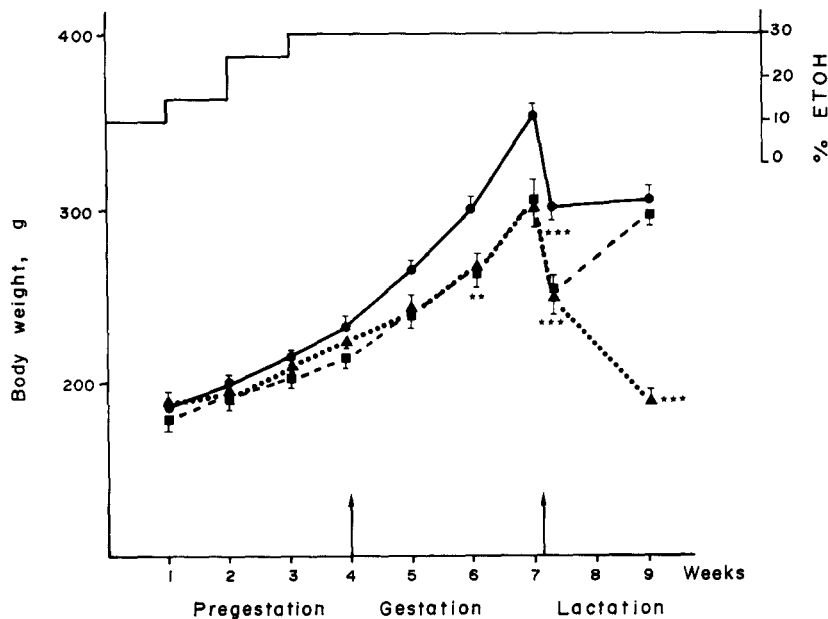


Fig. 3. Body weight in the rat under alcohol treatment. Specifications as in Fig. 1.

parturition; during this period, total caloric intake in ethanol rats was significantly higher than in their controls (Fig. 2). At parturition, total caloric intake decreased more in mothers under alcohol treatment than in controls and immediately after parturition, there was an initial rise in the first group lasting only until the 4th day of lactation, decreasing later to values significantly lower than in controls. Total daily caloric intake was completely normalized during lactation in the alcohol-deprived mothers and their values did not differ from those of controls during the post-partum period (Fig. 2).

In spite of the changes in daily total caloric intake, body weight did not differ between alcohol-treated and control rats until the sixth week of treatment, corresponding to the 15th day of gestation (Fig. 3). From this time on, body weight values in alcohol-treated mothers were significantly reduced compared with controls and this lower body weight in the alcohol-treated mothers became more marked as lactation progressed. In the alcohol deprived mothers, body weight returned to normal by the 15th day of lactation (Fig. 3).

Parturition occurred in controls at day 22.1 ± 0.1 of gestation and was delayed in mothers under alcohol treatment (at day 23.3 ± 0.2 , $P < 0.001$ compared with controls). This delay was not diminished when alcohol was withdrawn from mothers after the 21st day of gestation, parturition occurring at day 23.0 ± 0.2 ($P < 0.001$ when compared with controls and not significant when compared with mothers receiving alcohol for the whole period).

As shown in Table 1, litter size did not differ between the three groups of animals studied, but body weight at birth was significantly reduced in offspring from both alcohol-treated and alcohol-deprived mothers. By the 8th post-natal day, body weight was greatly reduced in offspring from alcoholic mothers compared with controls (Table 1). In offspring of alcohol-deprived mothers, body weight at 8 days of age was also lower than in controls but was twice as high as in those from alcoholic mothers. Body length at 8 days of age was significantly less in offspring from alcohol-treated mothers than in litters of either controls or alcohol deprived mothers. There were no differences in body length in the two latter groups but skeletal maturity values were significantly lower in the offspring of alcohol-deprived mothers than in controls at 8 days of age (Table 1). At 15 days of age, the sharp reduction in body weight and length in offspring of alcohol-treated mothers was maintained, while litters of alcohol-deprived mothers did not differ from controls in either parameter (Table 1). They did, however, remain retarded in their skeletal maturity and this value was statistically significant when compared with age matched controls (Table 1). During the 15 days of post-natal life, there was no offspring mortality in controls while it was 27% in offspring of alcohol-treated mothers and 3% in those from alcohol-deprived mothers.

Circulating metabolites and liver glycogen concentrations were measured in offspring at the 15th day of age and values are summarized in Table 2. Offspring from alcoholic mothers show significant reductions in both blood glucose and liver glycogen concentrations and increases in blood beta-hydroxybutyrate concen-

Table 1. Effect of maternal ethanol ingestion in the rat on litter size and growth parameters in offspring

	Litter number	8th day of age			15th day of age			
		Body weight at birth (g)	Body weight (g)	Body length (cm)	Total skeletal maturity score	Body weight (g)	Body length (cm)	Total skeletal maturity score
Control mothers ¹	10.2 ± 0.6^a	6.6 ± 0.2^a	20.3 ± 0.3^a	7.8 ± 0.1^a	34.0 ± 0.2^a	33.7 ± 0.6^a	9.6 ± 0.1^a	65.1 ± 0.2^a
Alcohol mothers	9.0 ± 0.7^a	5.0 ± 0.1^b	9.9 ± 0.4^b	7.3 ± 0.1^b	—	11.8 ± 0.4^b	7.3 ± 0.1^b	—
Alcohol-deprived mothers from day 21 of gestation	8.8 ± 0.6^a	5.3 ± 0.1^b	18.8 ± 0.2^c	7.6 ± 0.1^a	31.6 ± 0.3^b	34.6 ± 0.3^a	9.3 ± 0.1^a	55.4 ± 0.6^b

¹Data are given as means \pm SEM of 20–40 animals/group. Body length correspond to body size without tail.

^{a,b,c}Values in the same row not followed by the same superscript letter differ significantly, $P < 0.05$.

Table 2. Effect of maternal ethanol ingestion in rat on offspring metabolic parameters at the 15th day of age

	In blood			
	Glucose (mg/dl)	Acetoacetate (μ mol/dl)	β -hydroxybutyrate (μ mol/dl)	Liver glycogen (mg/100 mg)
Control mothers ¹	123.8 \pm 5.4 ^a	22.2 \pm 2.0 ^a	128.3 \pm 10.2 ^a	2.67 \pm 0.27 ^a
Alcohol mothers	93.8 \pm 4.5 ^b	23.1 \pm 5.3 ^a	214.1 \pm 31.0 ^b	0.18 \pm 0.01 ^b
Alcohol deprived mothers from day 21 of gestation	117.5 \pm 5.7 ^a	33.2 \pm 3.5 ^b	196.6 \pm 28.7 ^b	1.24 \pm 0.61 ^c

¹Data are given as means \pm SEM of 8–10 rats/group.

^{a,b,c}Values in the same row not followed by the same superscript letter differ significantly, $P < 0.05$.

trations, as compared with controls. In the offspring of alcohol deprived mothers, there were significant reductions in liver glycogen concentrations and increases of both acetoacetate and beta-hydroxybutyrate concentrations in blood compared with control values (Table 2).

DISCUSSION

The experimental protocol used in the present study for prolonged ethanol treatment of the pregnant rat was similar to those employed by others (Abel & Dintcheff, 1978; Lee & Leichter, 1980; Jones *et al.*, 1981). Pair-fed control animals were not used because no differences in parameters of offspring from *ad libitum* and pair-fed control mothers have been reported (Abel & Dintcheff, 1978; Lee & Leichter, 1980; Jones *et al.*, 1981). The present investigation demonstrate that when non-pregnant rats were exposed to progressively increasing dose of ethanol in the drinking water, there was a voluntary reduction in the amount of daily liquid ingestion which was paralleled by reduction in food intake causing decreased total caloric intake which did not affect body weight. This was probably due to a compensatory reduction in the utilization of fuels, as it is well known that one of the main consequences of ethanol metabolism is a reduction of the consumption of physiological substrates (for a recent review, see Herrera & Llobera, 1981). This condition rapidly changes when rats are mated and become pregnant. Gestation itself is a well known spontaneous stimulus for hyperphagia in the rat (Knopp *et al.*, 1975; Kumaresan & Turner, 1968; Champigny *et al.*, 1980). In our study in alcohol-treated rats, daily food and fluid intake rose during pregnancy, producing a daily caloric intake even greater than in controls during the same gestation time. This finding differs from our previous studies in which daily caloric intake during gestation in alcoholic rats was the same (Herrera & Llobera, 1981) or even slightly reduced (Mena *et al.*, 1982) compared with controls. In these experiments, however, alcohol treatment was initiated from mating time, while in the present study gestation started four weeks after initiation of alcohol treatment, probably causing addiction in the rats. The rise in total caloric intake in the pregnant alcohol-treated rat was not enough to pre-

serve her body weight increase which was significantly lower than in controls from the second week of gestation (Fig. 3). This difference became greater as gestation advanced and must be the result of both a reduced increase in conceptus weight due to the negative effects of maternal alcohol ingestion of fetal growth rate and to an inability of the alcohol-treated mother to build up her own stores as normally occurs in the rat at late gestation (Herrera *et al.*, 1969; Knopp *et al.*, 1970). This interpretation agrees with the reduced body weight of both alcohol-treated mothers and their offspring at parturition in spite of the fact that this occurs later than in controls, due to the well known effects of alcohol delaying labor (Abel & Dintcheff, 1978; Abel *et al.*, 1979; Abel, 1981).

The rise in food intake which occurs with lactation in control mothers is small and temporary in alcohol-treated, producing a marked body weight loss during the first 15 days of lactation. In these animals, milk production is probably reduced as demonstrated in other studies of alcohol ingestion in lactating mothers (Cobo, 1973; Rosett, 1979). The lack of availability of milk for the offspring of rats receiving alcohol while lactating must be the main factor causing the severe denutrition observed in these pups at the 15th day of age and manifested by their retarded growth development and their significant depletion of liver glycogen stores. Blood ketone body concentrations in these pups were slightly augmented as could be expected due to increased fatty acid breakdown produced by their subnutritional state. This situation, however, was similar to the condition produced by prolonged starvation in adult rats (Herrera & Freinkel, 1968) in which circulating ketone body levels were lower than in animals undergoing shorter periods of food deprivation, due to their lack of lipidic stores to be mobilized and oxidized. This explanation probably applies also to pups from alcoholic mothers, in which, in addition to a lack of lipidic stores, there is a drastic reduction in available milk, known to be the main lipidic source in the suckling rat (Dymska *et al.*, 1964; Aranda *et al.*, 1973).

When alcohol was withdrawn from mothers before parturition, they completely regained normal food and water intake values and body weights were also normal in both mothers and offspring at the 15th day post-partum, indicating that milk production in the mothers had also returned to normal. In spite of recovery of body size and weight in offspring of alcohol-

deprived mothers, skeletal maturation was still impaired, in agreement with Lee & Leichter (1980), which was consistent with the hypothesis that maternal alcohol consumption adversely affects the regulatory mechanisms for growth during embryonic development, this effect persisting after birth. Some metabolic disturbances are also persistent after birth in the offspring of mothers given alcohol prior to and throughout gestation, but not throughout lactation, as shown by the decrease in liver glycogen and rise in circulating ketone bodies in these pups at the 15th day of age. The changes were similar to those we previously reported in fetuses of alcoholic mothers at the 21st day of gestation (Herrera & Llobera, 1981). Further studies should be performed to determine whether the maintained metabolic disturbances and the retarded skeletal maturation in offspring of alcohol deprived mothers are interrelated. This is of interest as metabolic changes could be used for early diagnosis of the fetal alcohol syndrome.

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