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EFFECTS OF CHRONIC ETHANOL INGESTION ON CIRCULATING METABOLITES AND LIVER COMPOSITION IN THE LACTATING RAT*

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Abstract—1. A model of chronic ethanol administration has been used to study the effects of chronic ethanol consumption on the general metabolism of lactating rats on day 15 after delivery.

2. We have studied the effects of ethanol on calories, food and fluid intake, body weight, circulating parameters such as glucose, glycerol, free fatty acids (FFA), triacylglycerols (TAG), amino acids (AA), ketone bodies, insulin and ethanol levels and liver composition.

3. Chronic ethanol consumption markedly increases the levels of circulating B-OH-butyrate (B-OB-B), glycerol and FFA, while those of acetoacetate (AcAc), glucose, insulin and TAG remain constant. With the only exception of an increase in Glu + Gln levels, plasma AA decrease in the alcohol-treated rats, the change being significant for Ala, Pro, Lys, Arg, Val, Phe and 4-OH-proline.

4. In the liver ethanol treatment causes an increment in TAG concentration and a decrease in glycogen content.

5. In conclusion, chronic ethanol consumption produces notable alterations in the metabolism of lactating rats, which may diminish the efficiency of lactation, influencing milk production and, therefore, the pups' development.

INTRODUCTION

Since the 70's it has been known that alcohol, when ingested at gestation, may result in anomalies in the foetus called the Foetal Alcohol Syndrome (FAS) (Jones *et al.*, 1973; Krous, 1981). However, there is little information on the effects of alcohol ingestion during lactation. This period involves an important metabolic and physiological effort on the part of the dam since she has to produce the milk necessary to nourish her pups (Cañas *et al.*, 1982). Therefore, any alteration in the efficiency of this period will affect the normal pup development. There are some indications that this occurs when ethanol is ingested. It has been shown that rats treated with ethanol from delivery and during lactation present a delay in corporal growth (Abel, 1974; Fernandez *et al.*, 1982), which may affect the optimal milk production (Abel, 1978). It has also been observed that ethanol metabolization is faster in lactating dams than in virgin animals (Abel, 1979). The metabolic alterations which may accompany these effects in lactation have not been examined in detail until now, which is surprising considering the importance of this period.

For these reasons, the present study was carried out to determine the effects of chronic ethanol intake on lactating rats by determining the caloric intake and several metabolites both in circulation and the liver.

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MATERIALS AND METHODS

Female Wistar rats from our colony weighing initially 120 ± 10 g were maintained under automatically controlled temperature (23 ± 1°C) and 12 hr light–dark cycles. Animals were randomly divided into 2 groups: (1) Ethanol-treated rats which received ethanol diluted in the drinking water: 10% the first week, 15% the second week, 20% the third week and 25% the fourth week (v:v). After the fourth week of treatment rats were mated with untreated males. Impregnation was determined by daily vaginal smears. Throughout gestation and lactation (until day 15 after delivery) the rats received the upper doses of ethanol (25%). Chow food and water were provided *ad libitum*. (2) Control rats (age- and sex-matched) received chow diet and water *ad libitum* and were handled in the same way as ethanol-treated rats. At delivery both groups were deprived of their pups which were exchanged for pups of untreated mothers born on the same day, there being 8 pups in each litter. This exchange of pups was carried out in order to avoid any interaction of effects produced by an altered gestation in the alcoholic group with the stimulus-depending secretion of milk. All the rats were killed by decapitation on day 15 after delivery. Blood was collected from the neck wound in dry heparinized beakers, and simultaneously, a piece of liver was dissected and frozen in liquid nitrogen. Plasma was obtained by centrifugation and used to measure urea (Fawcett *et al.*, 1960), protein (Lowry *et al.*, 1951), FFA (Falholt, 1973), TAG (Ramirez *et al.*, 1983a) and insulin (Heding, 1972) concentrations. Plasma aliquots were deproteinized in cold acetone (Arola *et al.*, 1977) and used for the determination of individual AA (Pastor-Anglada *et al.*, 1984). Whole blood was deproteinized (Somogyi, 1945) and used for the enzymatic determination of glucose (Hugget *et al.*, 1957), glycerol (Eggstein *et al.*, 1974), AcAc and B-OH-B (Williamson *et al.* 1962). Blood samples were also collected directly in small tubes with thiourea and used for the quantification of ethanol by gas chromatography (Espinet *et*

al., 1984). The dry weight of the liver was determined after placing liver aliquots in an oven for 48 hr at 100°C. In liver, glycogen was estimated as glucose (Hugget *et al.*, 1957) after its purification and hydrolysis (Good *et al.*, 1933), and proteins were analyzed by the Lowry *et al.* method (1951). Liver TAG content was determined as previously described (Ramirez *et al.*, 1983b). Results are expressed as means ± SEM. Statistical comparisons were made by the Student's *t*-test.

RESULTS

In Fig. 1 the body weights of both control and ethanol-treated groups throughout the treatment period (4 weeks before impregnation until day 15 after delivery) can be seen. Whereas body weight was similar in control and ethanol groups during the pre-gestation period, there are significant differences in body weights from day 7 of gestation until the end of the studied period, the differences being more marked after delivery.

Figure 2 shows the food and fluid consumption per day throughout the treatment period for both groups. As can be seen, the alcohol-treated rats eat and drink significantly less than control rats throughout the studied period.

Figure 3 shows the total daily kcal intake by control and ethanol groups throughout the studied period. In the ethanol group, the total kcal values are the sum of solid-derived calories plus ethanol-derived ones. Total daily kcal present the same profile in both groups during the pre-gestation and gestation periods. Throughout lactation the alcohol-treated

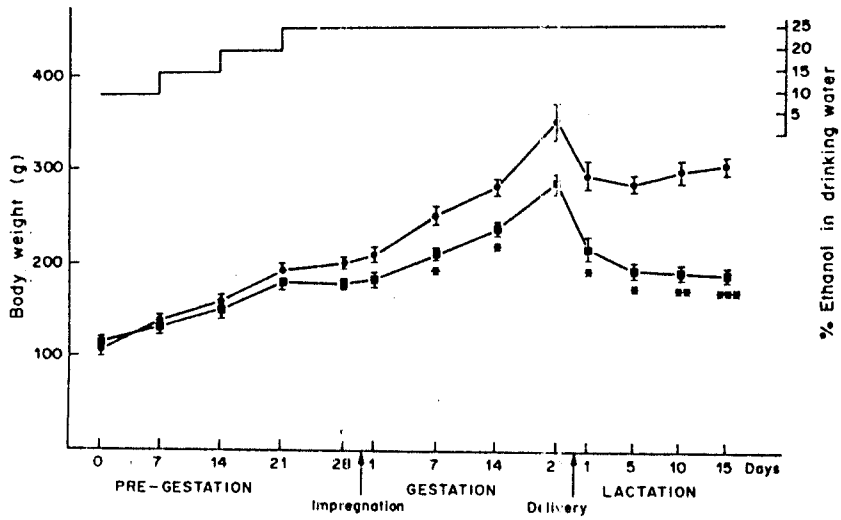

Fig. 1. Effect of ethanol on body weight in the rat. ●——● Control group, ■——■ Ethanol group. Values are means ± SEM of 8–10 animals. Statistical comparisons between control and ethanol groups are shown by: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

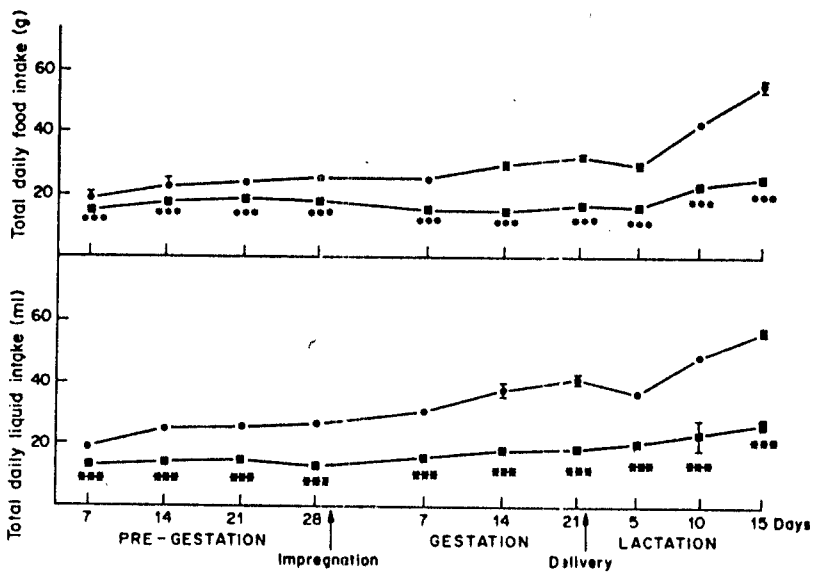

Fig. 2. Effect of ethanol on daily food and fluid intake in the rat. ●——● Control group, ■——■ Ethanol group. Statistical specifications as in Fig. 1.

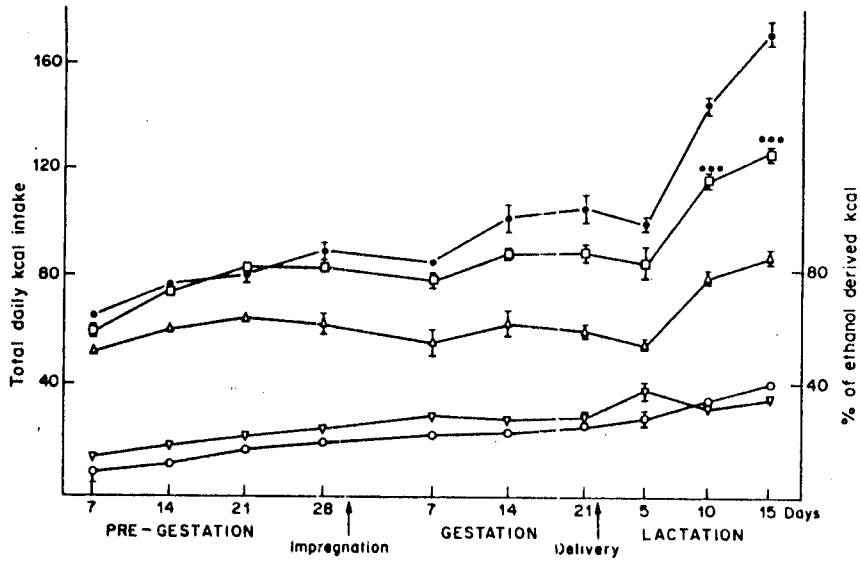

Fig. 3. Effect of ethanol on total daily calorie intake in the rat. ●——● Total daily calorie intake of control group, □——□ Total daily calorie intake of ethanol group, △——△ Food-derived calorie of ethanol group, ▽——▽ Ethanol-derived calorie of ethanol group, ○——○ Ethanol calorie as a percentage of total calorie intake of ethanol group. Statistical specifications as in Fig. 1.

group presents a marked reduction in its total daily kcal intake as compared to control rats. Figure 3 also shows the ethanol-derived calories as a percentage of the total value. This percentage slightly increases in parallel with the increasing doses of ethanol administered, achieving a value of nearly 40% of the total kcal at the end of the experiment.

Blood and plasma concentrations of several metabolites are listed in Table 1. Ethanol treatment markedly enhances blood B-OH-B levels but does not alter those of the AcAc, thus the B-OH-B/AcAc ratio increases dramatically in the ethanol-treated group. Ethanol has no effect on blood glucose concentration, whereas the ethanol-treated group presents a two-fold increase in blood glycerol values. Ethanol treatment does not affect the concentration of plasma TAG, proteins, urea and insulin, whereas FFA levels are significantly higher in the ethanol group. Plasma ethanol concentration is also shown in Table 1.

Table 2 shows the liver composition of the two experimental groups. A significant reduction in hepatic glycogen content in the alcoholic group can be seen, whereas there is no difference in either protein or water content in the liver. However, TAG concentration significantly increases.

Plasma AA concentrations are listed in Table 3. The individual values of AA generally tend to drop in the treated group, Ala, Pro, Lys, Arg, Val, Phe and 4-OH-proline being significant. The total AA value (as the sum of individual ones) also decreases.

DISCUSSION

The results obtained in this study show that, in agreement with previous reports (Herrera *et al.*, 1981; Ludeña *et al.*, 1983), throughout the adaptation period to alcohol given in the drinking water (pregestation period) the rats are able to counterbalance

Table 1. Effect of chronic ethanol treatment on several blood and plasma metabolite concentrations of lactating rats

	Control	Ethanol
Blood		
B-OH-Butyrate (μM)	17.4 ± 2.8	162.0 ± 45.9**
Acetoacetate (μM)	2.6 ± 0.2	3.0 ± 0.1
B-OH-B/AcAc	8.9 ± 2.0	64.8 ± 16.1**
Glycerol (μM)	47.9 ± 8.0	86.8 ± 5.2**
Glucose (mM)	5.0 ± 0.3	4.6 ± 0.1
Ethanol (mM)	—	28.2 ± 7.3
Plasma		
Insulin (μU/ml)	22.7 ± 3.7	13.8 ± 1.4
Triacylglycerol (mM)	1.0 ± 0.4	0.9 ± 0.2
Free fatty acids (μM)	174.0 ± 13.3	310.0 ± 48.7*
Proteins (g/l)	71.1 ± 1.6	70.0 ± 1.7
Urea (mM)	11.6 ± 1.0	11.1 ± 0.8

Results are means ± SEM of 5–8 animals. Statistical comparisons between control and ethanol groups are shown by: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

Table 2. Effect of chronic ethanol treatment on liver composition of lactating rats

	Control	Ethanol
Liver		
Glycogen (g/100 g.l.w.)	4.3 ± 0.1	2.5 ± 0.5***
Dry weight (%)	32.1 ± 1.5	29.9 ± 0.4
Protein (μg/mg)	162.0 ± 15.7	184.0 ± 10.7
Triacylglycerol (nmol/mg)	4.8 ± 0.2	10.7 ± 1.1***

Results are means ± SEM of 5–8 animals. Statistical comparisons between control and ethanol groups are shown by: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

Table 3. Effect of chronic ethanol treatment on plasma amino acid concentration (μM) of lactating rats

	Control	Ethanol
Alanine	1165.0 ± 36.3	461.0 ± 31.7***
Glutamate + glutamine	575.0 ± 21.5	832.0 ± 25.6***
Aspartate + asparagine	106 ± 16.2	120.0 ± 39.1
Glycine	425.0 ± 23.9	264.0 ± 14.1**
Serine	258.0 ± 45.8	222.0 ± 55.8
Threonine	280.0 ± 56.3	140.0 ± 21.1
Proline	351.0 ± 21.5	177.0 ± 7.8***
4-OH-proline	31.1 ± 3.3	21.0 ± 2.3*
Lysine	867.0 ± 79.7	368.0 ± 43.4**
Arginine	293.0 ± 24.4	158.0 ± 28.4**
Histidine	66.7 ± 9.6	47.2 ± 6.1
Citrulline	384.0 ± 110.0	281.0 ± 127.0
Ornithine	114.0 ± 15.5	74.0 ± 3.9
Valine	191.0 ± 14.3	142.0 ± 13.8*
Leucine + isoleucine	304.0 ± 22.3	241.0 ± 12.1
Cysteine	124.0 ± 31.1	107.0 ± 38.4
Methionine	25.9 ± 5.8	32.3 ± 8.7
Taurine	148.0 ± 13.0	140.0 ± 11.7
Tyrosine	108.0 ± 10.5	81.5 ± 16.0
Phenylalanine	94.0 ± 7.2	49.4 ± 5.2***
Tryptophan	100.0 ± 9.7	78.3 ± 24.2
TOTAL	6239.0 ± 250.0	4194.0 ± 104.0***

Results are means ± SEM of 5–8 animals. Total amino acid values are the sum of individual amino acid values for each rat. Statistical comparisons between control and ethanol groups are shown by: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

the amount of food-derived calories ingested with the ethanol-derived calories and, in this way, consume the same amount of total kcal as the control group. In pregnancy, the energetic demands, due to the foetus development, are more important than in virgin animals. The pregnant rats treated with ethanol continue to feed and drink less than the control pregnant rats. Although the amount of total kcal they ingested per day reaches the control levels, this is not enough to ensure, wholly, the typical increase in body weight of this period and, hence, the body weights between both groups begin to differ significantly. This different body weight is certainly not only due to a lower weight of the foetus, since after delivery, and throughout the lactation period, these differences become more evident. During lactation the energy required to assume optimal milk synthesis and production is so great that, possibly, the different quality of the ethanol-derived calories makes it more difficult to make up for the decrease in the food-derived calories ingested by the lactating alcoholic group. Moreover, this experimental group fails to maintain its levels of total calories ingested at the level of control lactating rats, becoming, only in this period, hypocaloric compared with the control group.

Neither blood glucose nor plasma insulin are significantly affected by chronic ethanol administration. This maintenance of blood glucose levels may be a consequence of an increase in hepatic glycogenolysis as the low glycogen content of liver suggests, although a potential decrease in glucose utilization by extra-hepatic tissue could be involved. This last suppposition can be proffered in view of the important increase in ketone body levels observed in the alcoholic group, since ketone bodies may be used as an alternative energetic source in the extra-hepatic organs (Robinson *et al.*, 1980). This increase in ketone body levels may be a secondary consequence of alcohol effects increasing adipose tissue lipolysis (Baraona *et al.*, 1979), as indicated by the increase in circulating FFA and glycerol. Ketonemia in the alcoholic group is a consequence of the sole increase of B-OH-B, while the AcAc remains constant, producing in this way a marked increase in the B-OH B/AcAc ratio. The cause of this greater ratio may be the effect of ethanol metabolization which produces a notable shift in the reducing power of hepatocytes by generating NADH (Sorrell *et al.*, 1979). On the other hand, the increased FFA reaching the liver may contribute to the development of the fatty liver (as indicated by the increase of the hepatic TAG concentration) due to a partially inhibited hepatic lipoprotein release by ethanol action (Baraona *et al.*, 1979).

Chronic ethanol treatment produces a marked decrease in plasma AA levels. This effect may be

consequence of decreased entry of AA into the organism due to a diminished intake and/or altered intestinal absorption as has been described in *in vitro* studies (Jacobs *et al.*, 1980). Nevertheless, *in vivo* studies suggest that ethanol would have no effect on the overall passage of AA through the intestine (Green *et al.*, 1981). Our results show that only three essential AA (Lys, Phe and Val) significantly decrease and that, moreover, both protein and urea plasma levels are unchanged after chronic ethanol treatment. These results suggest that the decrease in intake and/or absorption of AA cannot be the principal cause of the circulating AA decrease observed. However, it may be implicated. It has been described that ethanol produces a negative nitrogen balance by increasing lost urinary (and not fecal) nitrogen (Chapman *et al.*, 1979). This may be the consequence of accelerated amino acid metabolism, which may account for a decrease in plasma AA and an increase in plasma glutamine bearing in mind its role in inter-organ nitrogen transport (Aikawa *et al.*, 1973). This overall view on amino acid metabolism would not concur with the literature about the effects of ethanol on amino acid transport, which suggests that ethanol could cause an inhibition in amino acid transport systems, especially the A system (Rosa *et al.*, 1980) which is mainly implicated in alanine transport, this amino acid being the one which decreases more markedly. Nevertheless, it should be taken into account that very little information is available on *in vivo* effects of ethanol on amino acid transport (Chambers *et al.*, 1973). More detailed experiments are required to explain these possible discrepancies. The results obtained suggest that ethanol affects amino acid metabolism in the lactating rat in a more pronounced way than in other physiological states, even more than in the gestational period is suggested in a recent paper (Marquis *et al.*, 1984) where with a model similar to ours, applied to pregnant rats, they did not detect any dramatic effects of ethanol on plasma amino acid levels. Unfortunately, the circulating ethanol concentrations were not published and, therefore, it is difficult to compare the results.

Finally, we do not know how these metabolic alterations produced by ethanol consumption by the lactating mother may affect the normal mammary gland function and milk production. At present, we are examining these subjects since our results suggest that the negative consequences of alcohol in the gestational period for the foetus development may be increased by ethanol drinking in lactation.

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