EFFECTS OF PRENATAL OR POSTNATAL ETHANOL CONSUMPTION ON ZINC INTESTINAL ABSORPTION AND EXCRETION IN RATS

M. L. MURILLO-FUENTES, R. ARTILLO, M. L. OJEDA, M. J. DELGADO, M. L. MURILLO and O. CARRERAS*

Department of Physiology and Zoology, Faculty of Pharmacy, University of Seville, Seville, Spain

(Received 4 April 2006; first review notified 20 June 2006; in revised form 11 August 2006; accepted 11 August 2006; advance access publication 26 October 2006)

Abstract — Aims: The effect of ethanol consumption, either during the pregnancy or lactation period, on the altered metabolism of zinc is not well-defined; consequently, this study was performed to analyze the effect of chronic ethanol exposure on milk consumption, serum, milk, duodenal absorption, fecal and urinary excretion of zinc in dams and offspring during either gestation or lactation in the rat. A complementary study was performed regarding pregnancy outcome. We evaluated testosterone values, the offspring born/litter and several indices such as fertility, viable gestations and the survival index. Methods: To study the effect of chronic alcoholism during gestation or lactation separately, at birth control newborns were cross-fostered to ethanol dams (ED), and the offspring issued from the ethanol treated mothers were cross-fostered to control dams (CD). Thus, three experimental groups of offspring were formed: (i) control offspring receiving no treatment (CO); (ii) offspring exposed to ethanol only during gestation (GO); and (iii) offspring exposed to ethanol only during lactation (LO). All the results were compared with offspring pair-fed groups (PFO) born of the pair-fed dams (PFD).

Results: Duodenal absorption of zinc increased significantly in LO offspring when the substrate concentrations in the perfusion medium were 25, 75, and 150 μM. A higher faecal excretion in GO pups compared with those with LO exposure and control groups (CO and PFO). The urine excretion of zinc was higher for LO offspring with respect to the other three experimental groups (CO, GO, and PFO). Conclusions: Maternal adaptation resulted in zinc retention, adequate to meet the demands of pup’s growth in the face of a lower diet intake. The zinc status in pups is regulated by a higher absorption of zinc and intestinal conservation of endogenous fecal zinc after postnatal ethanol consumption. The increase in urinary zinc excretion could be responsible for decreased serum zinc. However, we found an increase in serum zinc probably due to an increase in the zinc absorption values.

INTRODUCTION

Alcoholics frequently show zinc deficiency (Prasad, 1991). Zinc (Zn) is a nutrient required for many proteins involved in DNA synthesis, protein synthesis, mitosis, and cell division and also plays an important role in gene transcription (Hambidge, 2000). Zinc is also a micronutrient crucial for normal growth and development. As a consequence, nature has developed an array of elaborated processes for the absorption, storage, and transport of zinc within the body, and a homeostatic balance between these mechanisms is essential for good health (Krebs, 2000).

Zinc is a cofactor for alcohol dehydrogenase (ADH) (Seyoum and Persaud, 1995), the ethanol-metabolizing enzyme. Ethanol-induced zinc deficiency could decrease ethanol metabolism, resulting in an increase in circulating and tissue ethanol levels.

Zinc metabolism is altered by ethanol at several levels, including reduced dietary zinc intake and increased zinc excretion in urine. During pregnancy ethanol consumption is known to be antagonistic toward offspring growth as well as the status of zinc in humans and rats and this is proposed to be due primarily to a transient fetal zinc deficiency (Carey et al., 2000). Lower plasma zinc levels are also present in infants with fetal alcohol syndrome, and increased urinary zinc excretion appears to be responsible for decreased plasma zinc concentrations (Assadi and Ziai, 1986).

Nutritional requirements during pregnancy and lactation are known to be greater than those of non-pregnant/non-lactating subjects. It is generally accepted that malnutrition accompanies alcohol abuse. Carey et al., 2003 demonstrated that supplementing the diet of pregnant mice with zinc at the time of ethanol exposure on G8 significantly decreases fetal teratogenicity and increases viability. Studies in pregnant and lactating primates have shown that zinc absorption/retention was higher in conditions of metal deficiency (Lonnerdal et al., 1990). In fact, zinc supplementation of ethanol-treated pregnant rats has indicated some protective influence against the embryopathic effects of ethanol in pregnant rats (Seyoum and Persaud, 1995), against cognitively impaired in ethanol exposure (Summers et al., 2006), also prevents alcoholic liver injury in mice (Zhou et al., 2005) by inhibiting the generation of reactive oxygen species (P450E1) and enhancing the activity of antioxidants pathways gene transcription (Hambidge, 2000).

It is well known that ethanol consumption damages the gastrointestinal tract and influences absorption of various metals. Studies on the effects of ethanol on zinc absorption are scarce. We were able to find a few studies on the effect of ethanol on Zn absorption in animals and in humans, with contradictory results; however Mc Donald and Margen (1980) reported a non-significant decreased intestinal absorption of zinc in alcoholic patients but Dinsmore et al. (1985) reported a lower absorption. Antonson et al. (1983) observed in rats that chronic ethanol feeding was associated with decreased ileal, but not duodenal absorption of zinc in vivo. However, in chicks, ethanol decreased duodenal absorption of zinc (Wilson and Hoyumpa, 1973). Recent rat studies reported an enhancing effect of chronic ethanol consumption on $6^Zn$ absorption calculated on the basis of the 3-day fecal pool (Coudray et al., 2000). Studies in vivo performed in our laboratory showed a significant increase in jejunal zinc absorption at 21 days postpartum in offspring rats after a prenatal and postnatal exposure to ethanol (Tavares et al., 1998).
The purpose of this study was to assess the effects of maternal long-term ethanol consumption on duodenal absorption of zinc in offspring at 21 days postpartum. Previously, our research group has studied the effects in the remaining intestinal segments (jejunum and ileum). Furthermore, to study the maintenance of zinc homeostasis in the offspring we determined the urinary and fecal zinc excretion and the levels of zinc in serum and maternal milk. Most studies of the zinc–ethanol interaction have focused on the end of gestation as a model of fetal alcohol syndrome. However, a fostering/cross-fostering model during lactation (on Day 2 after birth) proposed in this study allowed us to determine the zinc–ethanol interaction in utero or in the suckling period in offspring, separately. A complementary study was performed on the pregnancy outcome. We evaluated testosterone values, the offspring born/litter and several indices such as fertility, viable gestations and survival index.

METHODS

Animals

Male and female Wistar rats, weighing ~150–200 g, were randomized into three dams groups: Control (CD), ethanol (ED), and pair-fed dams (PFD).

CD: water and basic diet were given ad libitum during the entire experimental period.

ED: ethanol and basic diet ad libitum.

PFD: water and isocaloric diet with the diet of ethanol rats.

These animals were used as parents. Male and female rats were mated to obtain the first generation offspring. Pregnant rats were housed individually in plastic cages. The day of parturition was designated as Day 1 of lactation, Day 21 being the end of the lactation period. The offspring number was reduced to 6–8 per mother from the time of parturition. The experiments were performed on the offspring of three groups at 21d postpartum. During the suckling period, the pups had free access to the nipples.

At birth (second day postpartum because the 1 day of lactation period the dams rejected to the new infants), the pups were divided into four experimental groups:

CO: Control new-borns, born and breastfed by their control dams.

GO: Pups issued from ethanol treated mothers cross-fostered to control dams. Pups exposed to ethanol only during gestation.

LO: Control new-borns cross-fostered to ethanol dams. Pups exposed to ethanol only during lactation.

PFO: Pair-fed new-borns, born and breastfed by their pair-fed dams.

Animals were maintained under an automatically controlled temperature (22–23°C) and a 12-h light–dark cycle (9:00–12:00 h). Animal care complied with the Guide for the care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996).

Ethanol treatment

Ethanol was administered in tap water by a previously described method (Tavares et al., 1998). Briefly, alcohol treated rats were given 5% ethanol (v/v) ad libitum for 1 week (5.5 ± 0.2 g ethanol/kg/day), 10% ethanol during the second (7.8 ± 0.4 g ethanol/kg/day), 15% during the third (8.9 ± 0.4 g ethanol/kg/day), and 20% ethanol during the fourth week (16.6 ± 2.1 g ethanol/kg/day). A consumption of 20% was maintained for 4 additional weeks. The total calories at the end of the experiment was similar in the different groups (CD: 163.10 ± 11.44; ED: 150.11 ± 1.82; PFD: 155.97 ± 10.83). Ethanol-treated rats were mated. The presence of sperm in the vaginal smear the following morning denoted Day 1 of pregnancy.

Pregnant females were replaced individually in their cages and assigned again to 20% ethanol as the sole source of liquid with food ad libitum, during the pregnancy and lactation periods. Offspring received ethanol through the maternal milk route.

Diets

Diets were prepared according to ILAR (1979), which details the known nutrient requirements for most of the common laboratory animals (g/kg of diet): Casein: 200; Sucrose granulated: 510; Cornstarch: 140; Fiber, cellulose: 50; Corn oil: 50; AIN-76 mineral mix: 35 (Albus; Córdoba, Spain); AIN-76 vitamin mix: 10 (Cecofar, Seville, Spain); Choline bitartrato: 2; DL-methionine: 3; Zinc: 0.029. Diet ingredients, including mineral and vitamin components, were mixed and homogenized in the laboratory in a double-cone blender (Rest Haan, Germany). Diet offered to animals as pellets.

Gestational parameters

A complementary study was performed to understand the pregnancy outcome. We evaluated testosterone values, the offspring born/litter and other several indices. The serum testosterone was determined by an automatic immunoassay system 1235 AutoDelfia™. The results of indices are expressed as a percentage:

Fertility index (FI): number of viable gestation/number of animals.

Viable gestation index (GI): number of successful gestation/number of animals.

Survival index: number of born pups alive/total number of pups born.

Zinc absorption

This set of experiments was performed on the offspring 21 days after birth. All experiments were done between 9:00 a.m. and 13:00 p.m. Pups were anaesthetized with subcutaneous urethane 10%. The abdominal cavity was opened by a longitudinal incision and the duodenum was cannulated with polyethylene tubing. Inflow and outflow cannulas were tied into jejunum. Thus, a duodenal loop was isolated from the lumen. After cannulation, the loop was rinsed with 0.9 NaCl solutions and replaced inside the body wall and perfused as previously described by Ponz et al. (1979). A flow rate of 3.0 ml/min during a period of 5 min was used, for each concentration in all animals. Pups were maintained under controlled temperature (37°C) with a heating pad. Bile conduct was ligated to avoid the interference of the zinc entero-hepatic cycle.
Zn as ZnCl was dissolved in a Tyrode medium whose composition (in g/l) is NaCl (7.36), KCl (0.2), CaCl2·2H2O (1.36) HEPES (2.4), HEPES-Na (1.3), and ZnCl (0.068) at final concentrations of 25, 50, 75, and 150 μM. This solution was pre-warmed (37°C) before perfusion. Zn uptake was measured in different experimental animals. The perfusion time for all the pups studied was divided into an equilibrium period of 15 min at the beginning of the experiment and one period of 5 min for each substrate concentration.

Zn absorption was determined as the difference between the initial and the final amount of substrate obtained in the perfusates. Analyses of Zn concentrations in the perfusates were carried out by Atomic Absorption Flame Spectrometry (AAFS), using external calibration.

All the water used was purified in a Milli-Q system (Millipore Corporation) at a resistance of 18 MΩ/cm. Laboratory glassware was kept overnight in 5% (v/v) Nitric acid and then washed with deionized water and dried in a dust-free atmosphere. Reagents and Zn standard were all from Merck (Suprapur quality). The stock solution (100 mg/l) was prepared according to the PerkinElmer guideline.

Before analysis, the samples were diluted with Milli-Q water (1:5 v/v). Zinc concentrations were determined using a PerkinElmer model 3100 Spectrometer, with air-acetylene flame, 213.9 nm resonance lines, and 0.7 mm slit. The calibration curve was linear up to 1 mg/l.

Morphometric intestinal evaluation
At the end of perfusions, pups were sacrificed and the duodenal tissue was removed to determine their wet and dry weights. The tissue fractions were weighed, dried in an oven at 105 ± 2°C to constant weight (24 h) and reweighed, to obtain tissue dry weight. Water small intestine was calculated by the difference between tissue wet and dry weight. To determine the total serosa area, the outer circumference was measured according to Winne (1976).

Blood samples
Blood samples were taken from fasted and anesthetized rats (subcutaneous urethane 10% w/v) by cardiac puncture through the thorax and collected in tubes. Serum was prepared using low-speed centrifugation (Hettich EDA 35) for 15 min at 1300× g. Before analysis the samples were diluted with Milli-Q water (1:5 v/v), as indicated by Miller and Miller (1993). Serum zinc levels were obtained as described above by AAFS. Theses values were assayed in both dams and offspring.

Urine and faeces samples
At the end of the experimental period (21 days postpartum), the animals were housed individually in metabolic cages and deprived of food for 12 h to collect urine and faeces. We measured urine volume and the amount of faeces excreted within 12 h. Water content of faeces was determined by drying the material at 105 ± 2°C. An appropriate amount of this resulting material was converted to ashes at 450°C. The residue was diluted with 5 M-HCl, 1:10 v/v and 1/2500 v/v for urine and faeces, respectively. AAFS was used for zinc determinations. Theses values were determined in both mothers and offspring.

RESULTS

Body weight
At birth no significant differences were found between the two groups studied in body weight. Ethanol consumption during pregnancy didn’t cause alterations in body weight of pups at birth (Table 1). Body weight at 21 days was lower in offspring fed with ethanol mothers than in CO. LO offspring show a significantly lower body weight compared with the control groups (CO, PFO) and the GO at Day 21 of lactation (P < 0.001; Table 1).

Milk consumption
Table 1 show that milk consumption was significantly lower for alcohol-administered groups (GO and LO) compared with control groups (CO, PFO; P < 0.001). There were no significant differences between the two groups of pups exposed to ethanol.

Intestinal zinc absorption
Results regarding intestinal zinc absorption are shown in Fig. 1. Duodenal absorption of zinc was significantly increased in LO offspring when the substrate concentrations in the perfusion medium was 25 (P < 0.05) and 75 μM (P < 0.001) compared with control groups (CO, PFO) and at 25 and 75 μM concentrations (P < 0.001) compared with GO. However, similar results in zinc absorption for the CO and GO were found.

Fertility parameters
As shown in Table 2, there was a slight reduction, but not significant, for number of litter size in the ED and PFO groups.
The results are expressed as mean ± SEM. Differences are analysed by a multifactorial analysis of variance (ANOVA) followed by the Tukey’s test. GO versus CO: ***P < 0.001; LO versus CO: +++P < 0.001.

Table 1. Effects of pre or postnatal ethanol consumption on body weight and milk consumption in the offspring rats

<table>
<thead>
<tr>
<th>Offspring (n = 15)</th>
<th>CO</th>
<th>EO</th>
<th>PFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at birth (g)</td>
<td>6.76 ± 0.25</td>
<td>6.10 ± 0.13</td>
<td>6.55 ± 0.22</td>
</tr>
<tr>
<td>Milk consumption (g)</td>
<td>1.202 ± 0.01</td>
<td>0.69 ± 0.06</td>
<td>0.69 ± 0.06</td>
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</table>

Fig. 1. Duodenal zinc absorption in offspring (µM/cm²/5 min). The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by the Tukey’s test. Number of animals: n = 14 compared with the CD. In the PFD group there was a slight reduction, but not significant, of fertility index and that of viable gestation. Testosterone levels were significantly decreased in ED and in the PFD (P < 0.001).

Morphometric parameters

Results regarding intestinal parameters are shown in Table 3. No changes in intestinal parameters were observed between experimental groups of pups except for intestinal water content (%) and total intestinal length. We detected significantly lower levels of water content in LO pups compared with CO (P < 0.05). The total intestinal length in both ethanol groups (GO, LO) was decreased with respect to PFO.

Zinc distribution

ED and PFD consumed significantly less zinc at the end of the lactation period than CD (Table 4a). Results regarding serum zinc are shown in Table 4a. The zinc content in dam’s serum was similar in ED compared with CD group at the end of the lactation period, although a slight increase, but not significant, in zinc serum was observed after ethanol exposure. However, the zinc serum values in PFD groups were decreased with respect to CD and ED groups.

In dams, the faecal and urine excretion was significantly lower for ED (P < 0.01; P < 0.001 respectively) in pups exposed to ethanol postnatally compared those exposed postnatally to control diet (CD). The zinc milk content at the end of the lactation period in animals given the ethanol diet showed a significant increase with respect to CD and PFD (P < 0.001). However, no significant differences between the controls groups (PFD and CD) were found (Table 4a).

Serum zinc was increased in pups exposed to ethanol postnatally (LO) compared with pups exposed to ethanol prenatally (GO) or controls (CO, PFO). However, we found a higher significant faecal excretion (P < 0.001) in pups exposed to ethanol prenatally compared those exposed postnatally (LO) and control groups (CO and PFO). On the other hand, the urine excretion of zinc was highly significant for offspring exposed to ethanol lactation (LO; P < 0.001) with respect to the other three experimental groups (CO, PFO, and GO pups; Table 4b).

DISCUSSION

Previous studies of the effect of alcohol on gonadal functions in experimental animals (Van Thiel et al., 1975) demonstrated that pair-feeding alcohol to male rats and isocalorically maintained controls for 41 days produced significant damage...
Offspring (n=15) CO GO LO PFO
Serosa area of duodenum (cm²) 3.07 ± 0.15 3.49 ± 0.07 3.20 ± 0.08 3.33 ± 0.16
Total Intestinal length (cm) 56.34 ± 1.16 53.79 ± 0.81; *P < 0.001 54.22 ± 0.71; bP < 0.01 60.17 ± 1.18
Water small intestine (%) 77.40 ± 0.25 76.13 ± 0.01 74.62 ± 0.12; *P < 0.05; bP < 0.05 77.5 ± 0.01

The results are expressed as mean ± SEM. Mean differences were studied by using analyses of variance (ANOVA) followed by the Tukey’s test. ‘n’ indicate the number of animals in each group.
GO versus PFO: *P < 0.001; LO versus PFO: bP < 0.05 LO versus PFD: bP < 0.01; LO versus CO: *P < 0.05.

Table 4. Effects of ethanol on zinc consumption, zinc maternal milk, zinc serum, and zinc excretion in dams and offspring rats, at the end of the lactation and suckling period (21 d postpartum)

Dams (n=5) CD ED PFD
a. Zinc consumption (mg/day) 1.01 ± 0.02 0.8 ± 0.01; ***P < 0.001 0.71 ± 0.01; ***P < 0.001
Zinc serum (p.p.m.) 1.24 ± 0.08 1.41 ± 0.10 0.903 ± 0.03; *P < 0.05, *P < 0.001
Zinc urine (µg/12 h) 7.06 ± 0.65 3.04 ± 0.30; ***P < 0.001 4.05 ± 0.4; ***P < 0.001
Zinc faeces (µg/12 h) 655.4 ± 39.6 476.2 ± 43.2; ***P < 0.001 380 ± 32; ***P < 0.001
Zinc milk (p.p.m.) 58 ± 0.3 68 ± 0.4; ***P < 0.001 57 ± 0.5; ***P < 0.001

Offspring (n=15) CO GO LO PFO
b. Zinc serum (p.p.m.) 1.23 ± 0.07 1.26 ± 0.04 1.50 ± 0.11; *P < 0.05 bP < 0.01 1.07 ± 0.04 1.07 ± 0.04
Zinc urine (µg/12 h) 1.50 ± 0.23 1.61 ± 0.15; ***P < 0.001 3.72 ± 0.37; ***P < 0.001 1.1 ± 0.15; *P < 0.05 bP < 0.01
Zinc faeces (µg/12 h) 172.3 ± 13.7 226.1 ± 2.08; ***P < 0.001 1.29 ± 12.2; *P < 0.001 168 ± 13.2; *P < 0.001

The results are expressed as mean ± SEM. “n” indicate the number of animals in each group.
ED vs CD: ***P < 0.001; **P < 0.01; PFD vs CD: ***P < 0.001; **P < 0.05; ED vs PFD: *P < 0.001.
LO vs CO: * P < 0.05; ***P < 0.001; LO vs PFD: *P < 0.01; a P < 0.001.
GO vs LO: 1 P < 0.001; GO vs CO: ***P < 0.001; GO vs PFO: a P < 0.001.

In vivo studies of Gordon et al. (1982) showed that alcohol given in different ways, such as gavages, pair-fed liquid diets, and drinking water or through intraperitoneally, could decrease blood testosterone levels, in rat and humans. The present results show a significant decrease in the values of serum testosterone after ethanol and pair-fed treatments and could indicate decreased fertility.

Lactation is a major component of reproduction unique in mammals. A few studies have examined the effects of alcohol administration during lactation. In our study, ethanol consumption decreased significantly milk consumption in both prenatally and postnatally ethanol-exposed litters at 21 days postpartum. This unimpaired nursing may be partially responsible for the impairment growth observed in ethanol-exposed litters. Rats exposed to ethanol during gestation had normal birth weights. Litters of dams exposed to ethanol postnatally were growth-retarded, whereas litters of dams exposed prenatally to ethanol achieved normal growth at the end of suckling. Nevertheless, they showed a significant lower weight gain compared with the control group during the last week of suckling. These results are in accordance with other studies performed in rats (Maldaner et al., 1994) and in mice (Middaugh and Boggan, 1991). Our results in rats exposed prenatally to ethanol also indicated a significant lower weight gain at the end of suckling and, according to a previous report, it could show a tendency for subsequent retarded growth. Furthermore, the studies on pair-fed showed a descent in body weight. Ethanol and pair-fed provoked a maternal malnutrition, this fact modified the offspring body weights during the suckling.

Several studies have shown that rats prenatally exposed to ethanol take a longer time to attach the nipple (Barron et al., 1991), and are incapable of exerting adequate suckling pressure and have a reduced number of rapid rhythmic sucks per minute of suckling (Rockwood and Riley, 1986). Maternal alcohol intake during lactation greatly impairs milk production in rats (Tavares do Carmo et al., 1999). Alcohol has an adverse effect on prolactin (PRL) and oxytocin release, two hormones essential for normal lactation. Subramanian (1997) working with a chronically catheterized lactating rat model,
indicated that ethanol administration adversely affects pup milk intake and growth and Heil and Subramanian (2000) have also reported that alcohol inhibits suckling-induced PRL release. However, recent studies have suggested that inhibition of oxytocin, rather than PRL, may be the primary avenue by which alcohol induces growth retardation during lactation. The other possible mechanism involved in alcohol’s inhibition on milk secretion could be its effect on the structure and function of the mammary gland (Vilaro et al., 1989).

Adequate transfer of zinc to the neonate during lactation is essential for offspring survival. In spite of the significantly reduced dietary zinc intake in ethanol-treated and in pair-fed dams, milk zinc levels significantly increased by ethanol treatment. It is known that milk concentration is maintained over a wide range of dietary Zn intake (Krebs, 1998) through coordinated regulation of mammary gland transporters (Kelleher and Lonnerdal, 2003). In this study we also analyzed the effects of ethanol on the distribution of zinc in dams that can become importantly affected by ethanol consumption. Data derived from zinc analysis showed that ethanol consumption didn’t significantly modify serum zinc levels although a slight increase was observed. However, malnutrition has an opposite effect of ethanol because the zinc serum value in pair-fed was decreased. The ethanol and malnutrition provoke, in dams, significant alterations in fecal and urinary excretion, leading to low urinary and fecal excretion. In humans, urinary zinc was significantly greater during wine and ethanol administration, suggesting that alcohol may affect the metabolism or renal conservation mechanism for zinc (McDonald and Margen, 1980). Low serum and increased zinc wastage are well-known features in alcoholic cirrhosis in humans (Rochi et al., 1994, Poo et al., 1995, Rodriguez Moreno et al., 1997); although later studies by the same authors have reported that ethanol administered during 8 weeks with a nutritionally adequate diet, a 36% (as energy) ethanol containing isocaloric diet, does not alter zinc serum, leading only to a slight, insignificant decrease in zinc excretion (Gonzalez Reimers et al., 1998). These studies are in accordance with our results and we concluded that malnutrition is responsible for the decrease in zinc excretion. Studies performed on mice giving ethanol intragastrically for 5 days in a dose of 2 g/kg of body weight observed an increase in zinc concentration blood serum (Florianczyk, 2000). Findings of another study (Coudray et al., 2000) showed no significant differences in fecal and urinary zinc after a long-term ethanol exposure (6% v/v during 1 month) in rats. Nevertheless, other recent investigations reported after long-term ethanol consumption (10% w/v) a decrease (52%) in the urinary excretion of zinc, whereas fecal excretion was increased (Brzózska et al., 2002). The findings of these studies are thus conflicting owing to the different animal models and the ethanol treatment used. Moreover, we performed the analyses during the suckling period, lactation posing a significant threat to maternal zinc homeostasis.

Zinc intestinal absorption has been studied extensively but the variety of experimental approaches and designs used have yielded contrasting data in some aspects of zinc transport and the major intestinal site involved in the process. Recent studies in rats have concluded that intestinal transport of zinc occurs by a saturable process in the small intestine, more than one transporters were possibly involved in the process; that there isn’t a well-defined major sector in zinc transport and; finally, there is a dependence on the concentrations tested (Condomina et al., 2002). The results of the present study and of a previous report performed in our laboratory (Tavares et al., 1998, and not reproduced here) suggest that the major intestinal site involved in the process in offspring rats is the duodenum. Our results showed an increase in duodenal zinc absorption in offspring exposed to ethanol during lactation compared with the other three experimental groups, which preferably occurred at 75 µM concentration. Furthermore, a recovery of zinc absorption at 150 µM to basal values was found, probably related to the higher zinc milk concentration in ethanol dams and the following saturation of transporters. These results indicate that ethanol and no the malnutrition is responsible for the alteration in zinc absorption.

Morphological and biochemical alterations of the mucosal intestinal cell can be partially responsible for the altered absorption of zinc. Recently, animal studies have also demonstrated that ethanol in utero induces epithelial cell damage (Estrada et al., 1996; Camps et al., 1997) and altered kinetics in the developing rat intestine (Estrada et al., 1996). In this sense, ethanol may damage the intestinal cells and increase the space between the cells where paracellular passive absorption may increase (Persson et al., 1990). Interestingly, Buts et al. (1992), using a similar experimental model of ethanol administration only during pregnancy, have shown that postnatal maturation of the small intestine in the ethanol offspring was depressed during the early nursing period, even though ethanol had been withdrawn at birth, although it returned to control by Day 15 postpartum. An immature aspect of the enterocytes that persisted until weaning was also found in this study. Our results showed no ethanol effects in serosa area of duodenum and total intestinal length at the end of suckling, whereas lower water content was observed in pups exposed to ethanol postnatally because of an ethanol effect. Prolonged ethanol ingestion results in altered water–electrolyte homeostasis and induces body dehydration (Carney et al., 1995). Actually we concluded (Garcia-Delgado et al., 2004) that maternal ethanol ingestion before and during gestation and suckling affects the renal function of the offspring, up-regulating renal AQP2 expression by an AVP-independent mechanism. The malnutrition increased the total length; however the ethanol decreased it. In conclusion, the effects of ethanol and malnutrition on intestinal length are contradictory.

Intestinal in vivo studies in rats fed the Lieber–Decarli liquid diet showed that net ileal absorption of zinc was reduced by 16%, whereas duodenal absorption was unaffected by chronic ethanol ingestion (Antonson and Vanderhoof, 1983). Studies performed in our laboratory to examine the effects of prenatal and postnatal exposure to ethanol in vivo absorption of zinc showed a significant increase in jejunal zinc absorption at 21 days postpartum (Tavares et al., 1998). Recent studies on animals reported an enhancing effect of chronic ethanol consumption (6% v/v during 1 month) on $^{67}$Zn absorption calculated on the basis of the 3-day fecal pool (Coudray et al., 2000).

The urinary excretion of Zn in pups was increased by postnatal ethanol consumption in relation to the other three
experimental groups. However, faecal excretion in pups exposed to ethanol prenatally increased compared with ethanol postnatally and control groups. The higher faecal zinc excretion in prenatal ethanol exposure (nursed by control mothers) is probably due to an increase in bile secretion observed in control dams (data not shown). Anyway, we have to take into account that faeces are the major routes of excretion of endogenous zinc. The enhancing effect of ethanol on zinc absorption observed in pups exposed to ethanol postnatally may be explained in the following ways: (i) Specific morphological and biochemical alterations on the gastrointestinal tract; (ii) The milk intake was lower in the pups from lactation group, and this could be compensated by the high levels of zinc in milk; (iii) Ethanol affects Zn clearance by increasing its urinary excretion, which in turn may increase intestinal absorption of zinc.

In summary, data derived from zinc analysis showed that after postnatal ethanol consumption zinc status was regulated by a higher absorption of zinc and intestinal conservation of endogenous faecal zinc. The increase in urinary zinc excretion could be responsible for decreased serum zinc. However, we found an increase in serum zinc probably due to an increase in the zinc absorption values. Further studies are needed for a better understanding of ethanol effects during pregnancy or lactation on Zn turnover.

Acknowledgements — Grant PM98-0159 supported this investigation from the Dirección General de Investigación Científica y Técnica (DGICYT).

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