Eur J Nutr 40 : 147–154 (2001)

ORIGINAL CONTRIBUTION

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Effects of maternal chronic alcohol administration in the rat: lactation performance and pup's growth

Summary A fostering/crossfostering analysis of the effects of maternal ethanol exposure on lactation performance and offspring growth was performed. Wistar rats were kept under one of the three experimental nutritional treatments: alcohol-treated (EG), pair-fed-treated (PFG) (as a nutritional control of alcohol-associated malnutrition), and control or normal diet (CG). Rats from the EG group were accustomed to increased amounts of ethanol (5 % during the first week to 20 % in the fourth week). The 20 % ethanol level was maintained throughout three additional weeks and during gestational and lactational period. Daily food intake, fluid consumption, body weight and gestational parameters were studied in control (CG), pair-fed (PFG) and ethanol dams (EG). At birth, half the litters were fostered to other dams of the same treatment (GLG) and half were cross-fostered to dams of the opposite treatment (GG, LG). No cross-fostering analyses were performed on the pair-fed group. Offspring body weight was controlled throughout lactation. Liver, kidney and spleen weights as well as milk consumption were also studied at the end of lactation period. In dams, a significant reduction of body weight was described throughout the suckling period. No ethanol detrimental effects were observed on body weight at birth, but in spite of a normal birth weight, alcohol during lactation was responsible for a growth deficit. Milk consumption was significantly reduced in offspring exposed to ethanol during gestation and/or lactation. Curiously, prenatal alcohol exposure affects adversely the suckling behaviour in pups at the time of weaning. In our study, alcohol treatment and malnutrition affects liver and spleen weights. However, malnutrition decreases spleen weights more than alcohol treatment. In the case of the kidney weights the alcohol decreases kidney weight more than malnutrition.

Collectively, the data from the present study show similar effects following pre/postnatal and postnatal alcohol exposure. The findings suggest that chronic alcohol administration during gestation and/or lactation adversely affects pup growth at weaning as indicated by its effect on milk consumption, pup and organ weight.

Key words Ethanol – Fostering/crossfostering – Gestation – Lactation – Nutrition – Offspring

Received: 3 January 2001
Accepted: 8 June 2001

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Introduction

Pre- and postnatal growth retardation is a characteristic feature of the fetal alcohol syndrome in both humans and laboratory animals (1–4). Since the first reports of fetal alcohol syndrome (FAS), thousands of studies have examined the effects of antenatal alcohol exposure in humans. Animal experiments also indicate that alcohol is a teratogen (5–8). The mechanism by which ethanol exerts its effect on fetal development is still unclear. At present, animal research indicates a multifactorial mechanism of the teratogenicity of alcohol resulting from nutrient deficiencies, fetal hypoxia and alterations in enzyme activities and cell function (9).

Chronic ethanol abusers are frequently malnour-

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ished, either generally or selectively. Ethanol is a relatively high-density source of calories. The caloric value of ethanol is thought to be "empty", in that it is preferentially utilised for energy. The high-energy value of ethanol leads to displacement of other foods and alters essential nutrient uptake (10). Even with adequate dietary intake, excessive consumption of alcohol may result in various vitamin and mineral deficiencies because of alcohol effects on absorption, transport, storage, metabolic activation and excretion of these substances (11, 12). A greater vulnerability of women to the development of alcoholic injury has been demonstrated in animals and humans (13, 14). Any decrease in the maternal transfer of nutrients will not only have negative consequences for the mother's own health, but will reduce nutrient availability for the fetus as well. The impact of poor nutrition on the developing fetus will be relatively greater because the fetus, with the higher metabolic rate needed to fuel its rapid growth, uses more nutrients per unit body mass than the mother (15). The capacity of the placenta to transport nutrients to the fetus is also reduced by alcohol consumption, thereby decreasing amino acid, glucose and other nutrient availability (16).

Ethanol may also impair lactational performance affecting mammary gland function and pup growth, which may add to the effects of ethanol during gestation (17). Animal studies have shown that alcohol-exposed rat pups take a longer time to attach to the nipple (18), are incapable of exerting adequate pressure and have a reduced number of rapid rhythmic sucks per minute of suckling (19). Moreover, chronic alcohol administration to lactating rats also affects suckling-induced prolactin release (20).

The present study was performed to determine the effects of maternal alcohol consumption during gestation and/or lactation, on suckling performance in rat pups and its implications on pup growth. Moreover, we examined the possible contribution of maternal malnutrition to evident growth reduction.

Materials and methods

Animals

Wistar rats weighing about 150–200 g were divided into three groups: control (CG), pair-fed (PFG) and ethanol (EG). The pair-fed group was used as control for the malnutrition associated with chronic ethanol intake.

Alcohol-treated rats were given alcohol in tap water by a previously described method (20). Animals had free access to both diet and drinking solution throughout the experimental period. Alcohol-treated rats were given "ad libitum" 5 % ethanol (v/v) in drinking fluid for one week, 10 % ethanol during the second, 15 % during the third and 20 % ethanol during the fourth week. Then a concentration of 20 % was maintained for 3 additional weeks. At the end of this period, the males and females in the ethanol group were mated to obtain the 1st generation offspring.

Pregnant females (F, n=12) were replaced individually in their cages and assigned again to 20 % ethanol in tap water as the sole source of liquid with food "ad libitum", during the pregnancy and lactation period (21 days).

Control rats received no treatment and were handled in the same way as the alcohol-treated ones.

Pair-fed rats were given the same amount of diet per day as consumed by the alcohol-treated animals.

Male (M=12) and female (F=12) rats were mated to obtain the 1st generation offspring. The presence of sperm in the vaginal smear the next morning denoted day 1 of pregnancy. Pregnant rats were housed individually. The day of parturition was designated as day 1 of lactation and day 21 as the final day of the lactation period. Parturition time and litter sizes were noted and offspring number was reduced to 6–8 per mother from the time of parturition. The experiments were performed on the 21st postnatal day. During the suckling period the pups had free access to the nipples.

To study the effect of chronic alcoholism during lactation or gestation separately, at birth (2nd day postpartum) control new-borns were cross-fostered to ethanol dams (EG), and the pups issued from the ethanol-treated mothers were cross-fostered to control dams (CG). Thus, five experimental groups of pups were created:

CG: Control pups receiving no treatment during gestation and lactation.
PFG: Pups receiving no alcohol treatment, but issued and fed by pair-fed dams.
GG: Pups exposed to ethanol only during gestation.
LG: Pups exposed to ethanol only during lactation.
GLG: Pups exposed to ethanol during gestation and lactation.

Animals were maintained under an automatically controlled temperature (22–23 °C) and a 12-hour light-dark cycle (9:00 to 21:00 h). Animal care complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington DC, 1996).

Diets

Diets were prepared according to The Council of the Institute of Laboratory Animal Resources (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; fibre, 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.

Diet ingredients including mineral and vitamin components were mixed and homogenized in the laboratory in a double-cone blender (Rest Haan, Germany). The diet was offered to the animals as pellets.

Coprophagia was avoided by placing wire nets over the cage floor. Daily caloric intake during lactation was estimated by the specified calorific value of the diet used (cal/kg) and the amount of food consumed by the dams, which was determined by daily weighing of offered and remaining food. The amount of daily fluid intake was also determined by volume difference between the offered and remaining liquid, and ethanol energy was estimated at 7.1 cal/g. Each measure was taken at 9:00 am to avoid changes due to circadian rhythms.

Gestational parameters

A complementary study was performed about pregnancy outcome. The results were expressed as indexes and given as a percentage:

Fertility index: number of viable gestation/number of animals

Viable gestations index: number of successful gestation/number of animals

Survival index: number of live born pups/number of born pups.

Milk consumption

The amount of milk consumed was estimated by subtracting the weight of the pups obtained just prior to returning them to the dam from the weight at the end of 30 minutes of suckling, as previously described by Subramanian (21).

Body and organ weight

Body weight was controlled weekly until the end of the experimental period. Each measure was taken at 9:00 am to avoid changes due to circadian rhythms. At the end of the suckling period, the pups were anesthetized with subcutaneous urethane 28 % w/v in saline, and liver, kidney and spleen were removed and weighed.

Statistical analyses

The values are given as the mean ± SEM. The indexes were given as percentage. Differences in means were studied by using analysis of variance (ANOVA), statistical significance established at $p < 0.05$ (Instat software program). When ANOVA resulted in differences, multiple comparisons between means were studied by a Tukey-Kramer test. Statistical differences were further assayed by the Kruskal-Wallis because most of the tests were nonparametrically distributed.

Results

DAMS

Gestational outcome

As shown in Table 1, there was a significant reduction for number of live-born pups (%) and litter size in the ethanol group compared to the two control groups. In the PFG group there was a slight reduction of fertility index and that of viable gestations.

Total food intake

As shown in Fig. 1, no significant differences were found among the three groups in total food intake. Food intake increases in all groups at the end of the lactation period.

Total fluid intake

As can be seen in Fig. 2, total fluid intake was also similar among the studied groups. The total fluid intake was slightly decreased in the EG group at the end of lactation period.

Table 1 Gestational parameters. Data are given as means ± SEM (n= indicates the number of animals in each group). Indexes are given as a percentage of 12 animals in each group

	CG	PFG	EG
Fertility index (%)	100	88.24	100
Viable gestations index (%)	95.24	82.35	100
Nº of born pups/litter	10.68±0.45 (n=19)	9.84±0.97 (n=14)	9.37±0.51* (n=27)
Survival index (%)	69.86	69.57	55.73

EG/CG: *p < 0.001

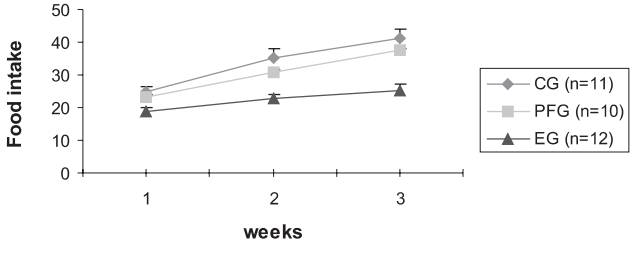

Fig. 1 Total food intake (g/rat/day) in nursing dams. Data are given as means ± SEM (n= indicates the number of animals in each group). Total food intake data are analysed by analyses of variance (ANOVA) followed by Kruskal-Wallis nonparametric statistic test.

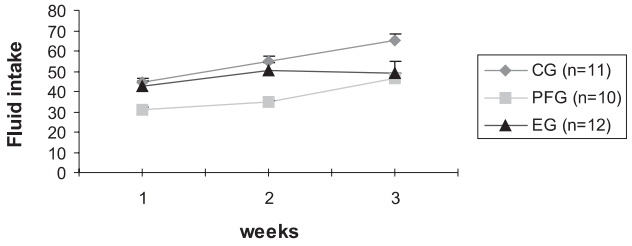

Fig. 2 Total fluid intake (ml/rat/day) in nursing dams. Data are given as means ± SEM (n= indicates the number of animals in each group). Total fluid intake data are analysed by analyses of variance (ANOVA) followed by Kruskal-Wallis nonparametric statistic test.

Total energy intake

The difference in the total energy intake (energy of food plus energy of fluid) was not significant among control (CG), pair-fed (PFG) and ethanol-fed rats (EG) (Fig. 3). As shown in Fig. 3, total energy intake in the alcohol group did not differ from that of controls, this balance being produced by the progressive increment in alcohol-derived energy and the correlated decrease in energy derived from food.

The evolution of rat's weight

The rats' body weight decreased in ethanol-fed rats during the 1st, 2nd and the 3rd week of lactation compared to control group ($p < 0.05$, $p < 0.001$, $p < 0.01$ respectively). A significant lower body weight was also found in the PFG group compared to CG ($p < 0.01$) during the first two weeks of lactation. However, there was no difference in body weight between EG and PFG animals (Fig. 4).

Offspring

Milk consumed by offspring during 30 min of suckling

The amount of milk consumed by pups during 30 min of suckling, obtained by weighing the litters before and at the end of the suckling period, is shown in Fig. 5. There were significant differences among groups. Comparisons between groups revealed that milk consumption was lower for alcohol administered groups compared to control (CG) and pair-fed (PFG) groups ($p < 0.001$). Among the three alcohol groups, milk consumption was lowered for the GLG group.

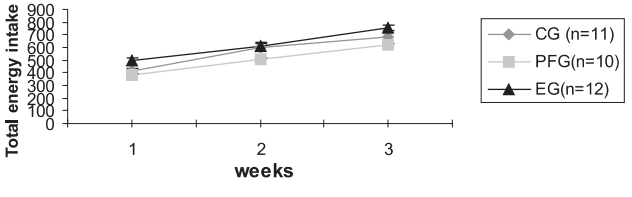

Fig. 3 Total energy intake (J/rat/day) of nursing dams. Data are given as means ± SEM (n= indicates the number of animals in each group). Total caloric intake data are analysed by analyses of variance (ANOVA) followed by Kruskal-Wallis nonparametric statistic test.

Body weight at birth and through suckling period

As shown in Fig. 6, no significant differences were found among the three groups of animals studied in body weight at birth. Nevertheless, a slight reduction in birth weight was observed in prenatal ethanol-exposed pups.

Values of offspring body weight during lactation are summarised in Fig. 7. Body weight at 7 days of age was lower ($p < 0.05$) in offspring issued and fed with alcoholic mothers (GLG) than in litters of either control or pair-fed mothers. At 14 and 21 days of age, the body weight also remained retarded in this group compared to control (CG), pair-fed (PFG) and the group exposed to ethanol only during gestation (GG) ($p < 0.001$, $p < 0.05$ and $p < 0.05$, respectively).

As shown in Fig. 7, body weight at 14 and 21 days postpartum of offspring exposed to ethanol only during

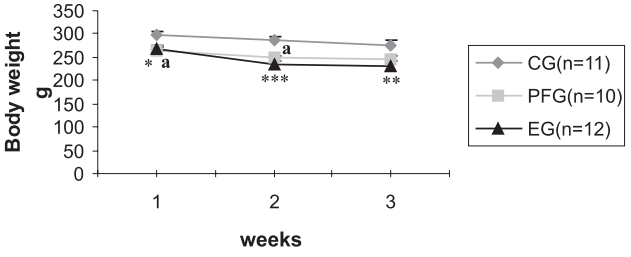

Fig. 4 Body weight (g) of nursing dams. Data are given as means ± SEM (n= indicates the number of animals in each group). Body weight data are analysed by analyses of variance (ANOVA) followed by the Tukey's test. *1st week: F: 6.842; P: 3.045. 2nd week: F: 12.765; P: 0.1459. 3rd week: F: 6.891; P: 0.4352.* a: significantly different from level in control group (CG) $p < 0.01$; *: significantly different from level in pair-fed group (PFG) $p < 0.05$; **: significantly different from level in pair-fed group (PFG) $p < 0.01$; ***: significantly different from level in pair-fed group (PFG) $p < 0.001$.

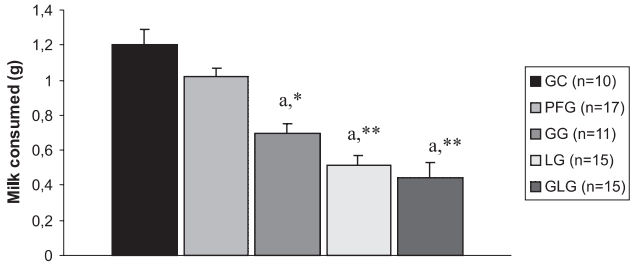

Fig. 5 Milk consumed (g) by offspring at 21 days postpartum. Data are given as means ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by Tukey's test (F: 22.792, P: 0.1427). a: significantly different from level in control pups (CG) $p < 0.001$; *: significantly different from level in pair-fed pups (PFG) $p < 0.01$; **: significantly different from level in pair-fed pups (PFG) $p < 0.001$.

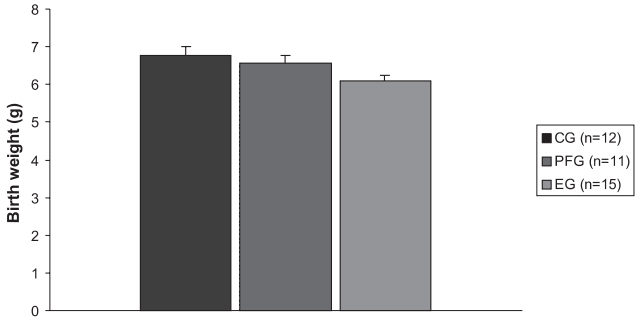

Fig. 6 Effect of maternal alcohol consumption on body weight of pups at birth (g). Data are given as means ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by Tukey's test.

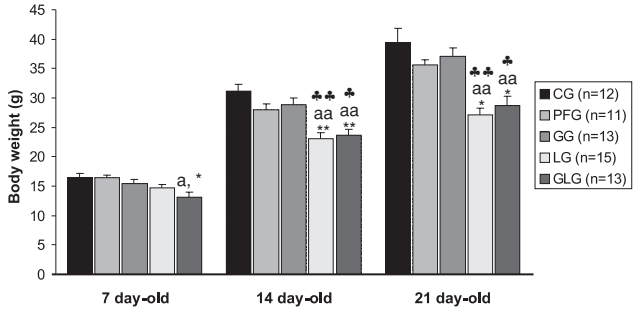

Fig. 7 Effect of maternal alcohol consumption on offspring body weight (g) throughout the suckling period. Data are given as means ± SEM and analysed by a multifactorial analysis of variance (ANOVA) followed by the Tukey's test: 7-day-old pup's weight (F: 3.238; P: 0.04623); 14-day-old pup's weight (F: 11.060; P: 0.9626); 21-day-old pup's weight (F: 8.747; P: 0.0017). a: significantly different from level in control pups (CG) $p < 0.05$; aa: significantly different from level in control pups (CG) $p < 0.001$; *: significantly different from level in pair fed pups (PFG) $p < 0.05$; **: significantly different from level in control pups (PFG) $p < 0.01$; ♣: significantly different from level in pups exposed to ethanol only during gestation (GG) $p < 0.05$; ♣♣: significantly different from level in pups exposed to ethanol only during gestation (GG) $p < 0.01$.

lactation (LG) was significantly diminished compared to CG, PFG and GG groups ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

Organ weight

As shown in Table 2, liver, kidneys and spleen weights were influenced by alcohol treatment in all groups of offspring studied. Alcohol treatment decreased liver weight in offspring at 21 days of age compared with control. Liver weight was significantly less in offspring exposed to ethanol only during the lactation period (LG) and throughout gestation and lactation (GLG) than in offspring control ($p < 0.01$; $p < 0.001$ respectively). Liver weight in PFG was also lower than in control pups ($p < 0.001$). Offspring exposed to ethanol (GG, LG and GLG) showed a decrease in kidney weights with respect to control groups ($p < 0.001$). For spleen weight at the end of the lactation period, there was a significant reduction

Table 2 Liver, kidney and spleen weight (g) of pups at 21 days of suckling period. Data are given as means ± SEM of 12 rats/group. Liver, kidney and spleen weight data are analysed by analyses of variance (ANOVA) followed by Tukey's test (liver F: 8.631; kidney F: 96.31; spleen F: 19.68)

	Liver	Kidneys	Spleen
CG	1.80 ± 0.11	0.514 ± 0.021	0.204 ± 0.013
PFG	1.33 ± 0.04^{aaa}	0.348 ± 0.011	0.091 ± 0.006$^{aaa, ♣♣}$
GG	1.53 ± 0.04	0.189 ± 0.004$^{aaa, ***}$	0.154 ± 0.008^{a}
LG	1.34 ± 0.08^{aa}	0.167 ± 0.010$^{aaa, ***}$	0.106 ± 0.007^{aaa}
GLG	1.27 ± 0.09^{aaa}	0.295 ± 0.015$^{aaa, ♣♣♣}$	0.188 ± 0.014 $^{***, bbb}$

a: significantly different from level in control pups (CG) $p < 0.05$; aa: significantly different from level in control pups (CG) ($p < 0.01$); aaa: significantly different from level in control pups (CG) ($p < 0.001$); ***: significantly different from level in pair-fed pups (PFG) ($p < 0.001$); ♣♣: significantly different from level in pups exposed to ethanol only during gestation (GG) ($p < 0.01$); ♣♣♣: significantly different from level in pups exposed to ethanol only during gestation (GG) ($p < 0.001$); bbb: significantly different from level in pups exposed to ethanol only during lactation (LG) ($p < 0.001$).

for the PFG group compared with the control group ($p < 0.001$). Consequently, spleen weight was lower in GG ($p < 0.05$), LG ($p < 0.001$) groups than in control offspring.

Discussion

In our study dams were given a 20 % ethanol solution during lactation. No significative differences in fluid, food and total energy intake were observed among the three groups of study (Figs. 1, 2 and 3). The rise in food and fluid intake, which occurs with lactation in control mothers, is small in alcohol-treated mothers. The intake of total energy was slightly higher in the alcoholic group during the lactation period. In line with these results, Gruchow et al. (22) observed that alcoholic calories are additive, at least in the diet of light drinkers; but, in the diet of moderate and heavy drinkers, alcoholic calories replace others sources of energy. Actually, Addolorato et al. (12) found that energy intake calculated as only food intake was lower in the alcoholics than in the controls, while the total energy intake, including ethanol consumption, was greater in the alcoholics. In previous studies, when rats were given ethanol as an isocaloric substitution for carbohydrates, a lower rate of weight gain could be seen (23). Our ethanol-fed and pair-fed animals also showed a reduction in body weight. This is probably due to a lower intake, although not significant, of food and fluid intake in both groups with respect to controls. No significant differences between pair-fed and ethanol-fed in the food intake were found. However, the total energy intake in the ethanol-exposed groups was due to the ethanol. Ethanol probably alters the metabolic rate, which may explain its failure to promote weight gain (24).

The data (Table 1) demonstrated adverse effects on

reproductive outcome although ethanol exposure had no influence on the number of dams carrying their pregnancy to term. Thus, litter size (number of pups per litter) and number of live-born pups were reduced by alcohol treatment. The high percentage of viable gestations found in ethanol-fed dams is also noteworthy. The efficient isocaloric adaptation may also be responsible for the high percentage of viable gestations in the alcoholic treated rats. These results are in line with a report by Testar et al. (25) and Buts et al. (7). In contrast to the above observations, Bond (26) observed that although alcohol administration influenced the percentage of successful gestations and incremented the percentage of neonatal mortality, it had no effect on litter size. This was probably due to the different ethanol treatments because the percentage of alcohol used by Bond was higher and was without a previous adaptation period.

Lactation is a major component of reproduction unique in mammals. Survival and optimal growth of all newborn mammals are dependent upon adequate milk secretion. Our animal studies indicated that alcohol consumption by gestating or nursing dams reduced milk intake by the suckling pups compared to the two control groups (Fig. 5). These results are in accordance with Subramanian (27). This author reported that milk consumed by pups during the 2-h period was lower for alcohol rats compared with control. Our findings in the pair-fed group suggested that maternal malnutrition is not primarily responsible for this alteration. Prenatal alcohol exposure has been associated with a variety of feeding and suckling deficits in humans (28). Alcohol-exposed infants took longer to attach and initiate suckling on a non-nutritive nipple. Animal studies have shown that alcohol-exposed rat pups take a longer time to attach to the nipple (18), are incapable of exerting adequate suckling pressure and have a reduced number of rapid rhythmic sucks per minute of suckling (19). While suckling deficits have previously been reported in rat pups following prenatal alcohol exposure, most of the suckling deficits were not observed in older pups (18, 19). For example, following prenatal alcohol exposure, normal attachment latencies were observed at 12–13 days of age. The adverse effect of alcohol on milk yield appears to be via alcohol inhibition of oxytocin release, affecting milk ejection from the mammary glands (29). Neonatal alcohol exposure interferes with suckling performance and these altered behaviours may contribute to the postnatal growth deficits that have been reported following alcohol exposure in utero (29). In recent studies, it has also been demonstrated that infusion of alcohol for 8 days from lactation day 5 to 12 affects milk consumption by the pups, litter growth and suckling-induced prolactin release (20). The data show that prenatal alcohol exposure affects adversely the ability to suckle at the end of lactation period. Actually Heil & Subramanian (30) examined how an extended period of suckling (120 min) affected suckling-induced prolactine release after chronic alcohol exposure. They found that after 120 min of suckling, prolactine release in dams receiving 2.0 g/kg alcohol was much higher than in control.

Body weight at birth did not differ among the groups of pups studied (Fig. 6). Studies of Gallo and Weinberg (31) have demonstrated no effect of perinatal exposure of ethanol administered in a liquid diet on offspring weights at birth. This result contrasts with the reduced body weight at birth found by other authors (17, 25) but is comparable to those observed by Bond (26). This difference may reside in the higher percentage of alcohol (25 %) used by Ludeña et al. (17) and Testar et al. (25), demonstrating that, in this case, fetal damage is dose-dependent. However, a significant reduction in body weight was observed at 14 and 21 days of age in the pups exposed to ethanol only during lactation (LG). Moreover, this body weight reduction was also evident in the pups exposed to ethanol during gestation and lactation periods (GLG) at 7 days of age, compared to the two control groups (Fig. 7). Buts et al. (7) demonstrated that prenatal exposure to ethanol decreases the body weight of pups at 5 and 10 days postpartum compared with controls. By day 9 of lactation and after a prenatal exposure to ethanol, Gallo and Weinberg (31) observed that control pups weighed more than both alcohol and pair-fed pups which were similar in body weight. Nevertheless, results are not comparable because in this study ethanol was withdrawn at birth and the exposure regimen was completely different from that used here. Our data, demonstrating adverse effects of maternal ethanol consumption on body weights of both the dam and the offspring during lactation, are consistent with previous data (20, 32). This lower weight is partly attributable to the reduced milk consumption as described above. Pups exposed to alcohol only during gestation (GG) have a similar body weight to both control groups, although these pups significantly consumed less milk at the end of lactation. However, this milk was without ethanol because the lactation was carried out by control dams. As shown in our results, alcohol content in milk is also responsible "per se" for this growth deficit observed following postnatal alcohol exposure. These data are consistent with the results reported by Lee and Leichter (33) and Singh et al. (34) who demonstrated that malnutrition in an alcoholic mother is not considered an important issue in growth retardation of the offspring. The lack of availability of milk for the offspring of rats receiving alcohol during lactation must be the main factor causing the severe denutrition observed in these pups.

There are few experiments attempting to assess potential postnatal maternal influence of prenatal ethanol exposure on development. To the best of our knowledge, effects of ethanol administration during lactation on

organ weights have not been previously reported. In our study, alcohol treatment and malnutrition affects liver weight (Table 2). Results also showed that pups exposed to ethanol during gestation and or during only lactation (GLG and LG) have a marked descent in liver weight at 21 days postpartum, respectively. Liver weight was also decreased in pair-fed groups. Curiously, liver weight of GG pups appeared to remain, although less, not significantly different from controls. It has been demonstrated previously that prenatal ethanol exposure induces a significant increase in the ratios of liver/body weight and hepatic protein level/body weight (35) and causes an increase in hepatocyte and mitochondrial volume (36). Another important effect of ethanol treatment on the newborn rat hepatocytes is a striking disorganization of the Golgi apparatus (36) and several dysfunctions in the glycoprotein metabolism of the organelle (35, 36). In line with these results, the kidney weight of pups exposed to ethanol only during gestation (GG), only lactation (LG) or during gestation and lactation (GLG) becomes significantly more affected than controls by the time of weaning. Malnutrition also adversely affects kidney weight, so that its weight is reduced, although in a minor proportion to ethanol exposed pups. Furthermore, kidney weight decreased in pups exposed to ethanol only during gestation or only during the lactation period compared to pair-fed ones. Therefore, we can deduce that alcohol decreases kidney weight more than malnutrition.

Previous works in rats have demonstrated the potential of paternal alcohol consumption significantly altering spleen weight in offspring at 21 days of age. An abnormal spleen/body ratio was observed in 15-day-old female offspring of beer drinkers during gestation (37). It is not possible to determine whether this difference is due to the differences in ethanol doses and exposure patterns (38). In our study, alcohol treatment and malnutrition also negatively affect spleen weight (Table 2). However, malnutrition decreases spleen weights more than alcohol treatment. On the other hand, the age of 90 days prenatal ethanol consumption does not affect kidney weight (39). However, in our case, prenatal effects were demonstrated at the weaning period in the kidney and spleen weights at 21 days postpartum. Previous studies established that chronic ethanol (25 % vol/vol) ingestion resulted in a significant reduction of liver weight (g) in offspring at 5, 10 and 30 days postpartum when compared to control (7, 40). All rats examined after birth were nursed by nontreated foster mothers, so that the effects of alcohol observed would be due exclusively to exposure in utero. In the present study, the data of liver weight are expressed as in previous studies, and we could also conclude that prenatal alcohol consumption decreases liver weight at 21 days postpartum. Moreover, comparisons between these data and the present ones are, therefore, problematic because ethanol treatment was withdrawn at birth, thus, the exposure regimen was completely different from that used here.

Collectively, the data from the present study show similar effects following pre/postnatal and postnatal alcohol exposure. Curiously, prenatal alcohol exposure adversely affects the suckling behaviour in pups at the time of weaning. Our results suggest that chronic alcohol administration during gestation and/or lactation adversely affects pup growth at weaning as indicated by its effect on milk consumption, pup and organ weight. These findings may have important clinical implications because they emphasize the potential significance of postnatal alcohol exposure.

Acknowledgments This investigation was supported by Grant PM98–0159 from the Dirección General de Investigación Científica y Técnica (DGICYT). Our thanks to Diane Haun Ph. D. for the professional English.

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