Alcohol & Alcoholism, Vol. 21, No 3, pp. 295–305, 1986
Printed in Great Britain

0309–1635/86 $3 00 + 0.00
Pergamon Journals Ltd

THE EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON PREGNANT RATS AND THEIR OFFSPRING

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(*First received* 9 *September* 1985; *received for publication* 12 *March* 1986)

Abstract — The effect of chronic ethanol intake during gestation was studied in rats fed a liquid diet in which ethanol provided 36% of the total calories. The animals were chronically alcoholised before mating, and the body weight gain and nutritional status during pregnancy were noted. Blood ethanol levels were measured during pregnancy and parturition. Specifically, we have shown that chronic ethanol intake during pregnancy lengthens the gestation period, decreases foetal viability, increases the placental weight and diminishes foetus, liver and brain weights, as well as the protein and DNA content of foetal brain. The reduced body weight of rats prenatally exposed to alcohol continued for the first two months of the postnatal period and was most apparent during lactation.

INTRODUCTION

It is known that ethanol consumption during pregnancy has deleterious effects on the normal course of foetal development, spanning the continuum from subtle growth and central nervous system alterations to death. The common pattern of dysmorphology has been termed the foetal alcohol syndrome (FAS) (Jones *et al.*, 1973). This dysmorphic picture is characterised mainly by pre- and postnatal growth deficiency, physical malformation, motor dysfunction and mental retardation (Streissguth *et al.*, 1980). Infants exhibiting the full syndrome are typically born to mothers who chronically consume large quantities of alcohol during pregnancy, and the severity of the embryo foetopathy is dependent on the stage of alcohol illness of the mothers (Majewski, 1981; Streissguth and Martin, 1983).

The specific mechanism(s) of alcohol teratogenicity remains unclear. In addition to clinical and epidemiological studies, numerous investigators have attempted to reproduce this syndrome in animals. Certain risk factors which are frequently associated with heavy alcohol intake (greater maternal age, lower social class, poor nutritional status, smoking etc.) can be eliminated in animal research and the direct effect of alcohol as a teratogen can therefore be studied.

The present study was conducted to evaluate the effect of chronic ethanol consumption by the rat during gestation, by simultaneous investigation of alterations in the alcoholic dams and their descendants. We have used an alcohol-containing liquid diet which, in the amount consumed, meets nutritional requirements and provides high blood ethanol levels, and which also facilitates the use of pair-fed controls. We have also assessed the nutritional status of the alcoholic dams, because the decreased food intake and accompanying malnutrition which are often associated with high alcohol consumption, could be crucial in studying the direct effect of alcohol *in utero*.

MATERIALS AND METHODS

Treatment of the animals

Adult virgin female Wistar rats, with an initial body weight of 200–225 g were used. All animals were acclimatised to laboratory conditions for at least one week before treatment. Animals were housed in groups of 4–5 each per

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cage and were maintained under controlled conditions of light (12 hr/12 hr), temperature (23 ± 1°C) and humidity (60%). The animals were divided randomly into three diet-based groups: (1) the alcoholic animals, which received an ethanol–liquid diet (5% w/v) in which ethanol provided 36% of the total calories (Lieber and DeCarli, 1976); (2) a pair-fed control group, which was given a similar diet, except that maltose–dextrin replaced ethanol isocalorically; (3) a second control group, which received a standard solid rodent chow (Sanders S. A., Madrid) and water *ad libitum*. The ethanol content in the alcohol liquid diet was increased gradually, from 1 to 5% (w/v) during a period of 1 week. Because rats dislike the taste of ethanol, a few drops of saccharin (110 mg/l of liquid diet) were added to both liquid diets. All groups were maintained on their diets for 4–5 weeks before mating. The animals were given fresh liquid diet each day and a record was kept during gestation of the daily food and/or fluid intake and body wt. Spillage and evaporation during each 24-hr period were negligible.

After 4–5 weeks on the above regimen, females in proestrus or initial estrus were placed overnight in cages with males of the same strain. The appearance of sperm in the vaginal washing the following morning defined day 0 of gestation. Pregnant rats were housed in individual cages with their corresponding diet available. At 8.00 a.m. on days 15, 19 and 21 of gestation, several rats from each group were killed by decapitation, the abdominal wall was immediately opened to expose the uterine horns, and the number of implantation sites, resorptions and malformed foetuses were counted and recorded. Foetal brains, liver and placentas were removed, weighed and kept at −30°C until processed.

Neonatal and developmental measures

Beginning on day 20 of gestation, other dams from each experimental group were routinely checked for births to determine both the exact gestation time and the litter size. Within 24 hr of birth, litters from alcohol-treated dams and pair-fed controls were removed from their physiological mothers, weighed, culled to 8–10 pups and assigned to a 'surrogate' control mother (chow and water *ad lib.*), which had delivered within the previous 24-hr period. The offspring were weaned at 25 days of age, males and females were housed in separate cages and were maintained on the rat chow and water diet. Body weights of animals were recorded at intervals until 4 months of age, at which time females were decapitated and brain weights were recorded.

Analytical methods

Alcohol was determined in tail-vein blood obtained from alcohol-treated dams on days 7, 15, 19 and 21 of gestation. At parturition, trunk blood of some decapitated newborns and blood from tail veins of the alcohol-treated mother were obtained simultaneously. Alcohol was determined in whole heparinised blood by head-space gas chromatography as described by Eriksson *et al.* (1977).

Brain DNA content was measured according to the procedure of Webb and Levy (1955). Protein was measured by the procedure of Lowry *et al.* (1951), using bovine serum albumin as standard. Liver glycogen was precipitated with ethanol after alkaline digestion and hydrolysed with sulphuric acid according to the procedure of Hassid and Abraham (1957). Water content of liver was determined as the difference between wet and dry weights.

Statistical evaluation

Student's *t*-test was used for statistical analysis. Data for offspring and foetuses are expressed as litter average.

RESULTS

Daily food consumption of the dams during the three weeks of gestation is shown in Table 1. The amount of food per dam increased throughout gestation for all groups, but on the day of parturition there was a 40% decrease. The caloric intake of the alcohol-treated and pair-fed animals was less than that for the chow–water group. When the results are expressed as kcal/body wt per day, the differences are more significant in the first week of gestation ($P \leqq 0.001$).

Total consumption and blood levels of ethanol during gestation and at parturition are shown in Table 2. When alcohol intake is

Table 1. Comparison of food consumption from different groups of pregnant rats during the three weeks of gestation (mean ± S.D.)

Groups	Daily caloric intake kcal/dam (kcal/kg body wt)			Daily protein intake g/dam (g/kg body wt)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Chow–water	67.39 ± 2.8 (312.0 ± 12.9)	73.54 ± 2.0 (308.3 ± 8.31)	77.02 ± 3.4 (264.3 ± 11.6)	2.95 ± 0.16 (18.32 ± 0.74)	4.30 ± 0.12 (18.05 ± 0.6)	4.51 ± 0.68 (15.5 ± 2.68)
Alcohol-treated	54.4 ± 6.2 (250.5 ± 23.6)**	64.5 ± 3 (285.2 ± 13)**	67.2 ± 2.8 (254.2 ± 13)*	2.33 ± 0.22 (11.1 ± 1.07)**	2.8 ± 0.13 (12.43 ± 0.5)**	2.91 ± 0.3 (10.12 ± 0.51)**
Pair-fed	55.5 ± 5.8 (250.5 ± 23.6)**	66.3 ± 2.8 (285.2 ± 13)**	71.1 ± 3.05 (254.03 ± 10.5)*	2.41 ± 0.22 (11.6 ± 1.3)**	2.92 ± 0.26 (12.43 ± 0.6)**	3.2 ± 0.3 (10.12 ± 0.51)**

Daily food intake was recorded during gestation. Each value represents the average ± S.D. for 30–40 pregnant rats. Significance of differences from chow–water group:* $P \leq 0.005$; **$P \leq 0.001$.

Table 2. Ethanol intake and blood levels in pregnant rats during gestation and parturition

Weeks of gestation		Daily ethanol intake g/dam (g/kg body wt)
1		2.7 ± 0.3 (13.2 ± 1.3)
2		3.2 ± 0.2 (14.4 ± 0.7)
3		3.5 ± 0.1 (12.9 ± 0.6)
Days of gestation		**Blood ethanol levels mmol/l (at 8.00 hr)**
7		12.7 ± 7.3
15		22.5 ± 12.1
19		23.1 ± 10.8
21		20.8 ± 4.9
At parturition	**Maternal levels**	**Newborn levels**
High levels	21.1 ± 4.6	23.5 ± 3.5 (4)
Low levels	4.3 ± 3.3	5.8 ± 2.2 (11)

Daily intake of an alcohol-containing liquid diet for 30–40 pregnant rats was recorded throughout gestation. Blood alcohol determination was measured as described in Materials and Methods. Numbers in parentheses in the lower part of the table represent the dams and their litter used. Each value represents the average ± S.D.

expressed as g/dam, the amount consumed increased during the three week period; however, because body wt was also increased markedly during the last week of gestation, ethanol consumption expressed as g/kg body wt was decreased. With this alcohol intake, pregnant rats showed blood alcohol levels of about 20–30 mmol/l, except in the initial days of pregnancy, when alcohol intake was lower. Interestingly, at parturition, 73% of alcohol-treated rats showed lower ethanol levels than during gestation; blood alcohol levels in newborn pups, however, were always 10–30% higher than in the corresponding mother.

Maternal weight gain during gestation is shown in Fig. 1. Weight gain by the two control groups was the same, until the 15th day of gestation, when the chow–water group showed a slight (though insignificant) increase over the pair-fed group. It must be noted, however, that despite strict isocaloric pair-feeding, the ethanol-fed animals did not gain as much weight as their pair-fed counterparts. The time-course of weight gain was also different; in both control groups, 50% of the total weight gain was achieved in 15 days of gestation, whereas in the alcohol-treated dams the weight gain was only 40% at 15 days and 60% in the last 6 days. At 21 days of pregnancy, the differences between maternal weight gain of alcohol-treated and either control group were significant ($P \leqq 0.001$), even taking into consideration the additional day of gestation for the alcohol-treated dams ($P \leqq 0.005$).

However, when maternal weight gain was corrected for the number of foetuses, the total increase in body weight was not significant at the end of pregnancy [alcohol-treated, 9.45 ± 2.13; pair-fed controls, 10.14 ± 1.97; chow-water controls, 10.99 ± 1.9 (means ± S.D. for 35–50 pregnant rats/group)].

Table 3 shows the effect of chronic alcohol consumption of foetal viability. Although the number of implants did not differ for the ethanol and control groups, the incidence of resorption in the pregnant dams was higher in alcohol-treated rats than in either control group at days 15, 19 and 21 of pregnancy. The decreased number of resorptions observed on days 19 and 21, as compared with day 15, probably reflects the fact that by days 19 and 21 the only vestige would be a small area of haemosiderin pigmentation at the original site of resorption. It is noteworthy that in one alcohol-treated dam on day 15 of pregnancy, all implantations were resorbed.

The effects of maternal ethanol intake on the development of bodies, brains and livers of foetuses, are shown in Fig. 2. Foetuses exposed prenatally to ethanol showed significantly reduced body, brain and liver weights, compared with either control group. Figure 2 also shows that the weights of the placentas from alcohol-treated dams were greater than in either control group. Since the two control groups did not differ significantly, it may be concluded that alcohol itself was responsible for these modifications.

Table 4 shows that protein, glycogen and water content of liver of 21-day-old foetuses from alcohol-treated rats were lower than those in control animals. Exposure *in utero* to alcohol also decreased the protein and DNA contents of foetal brains, although the latter were not significantly different when expressed per whole brain.

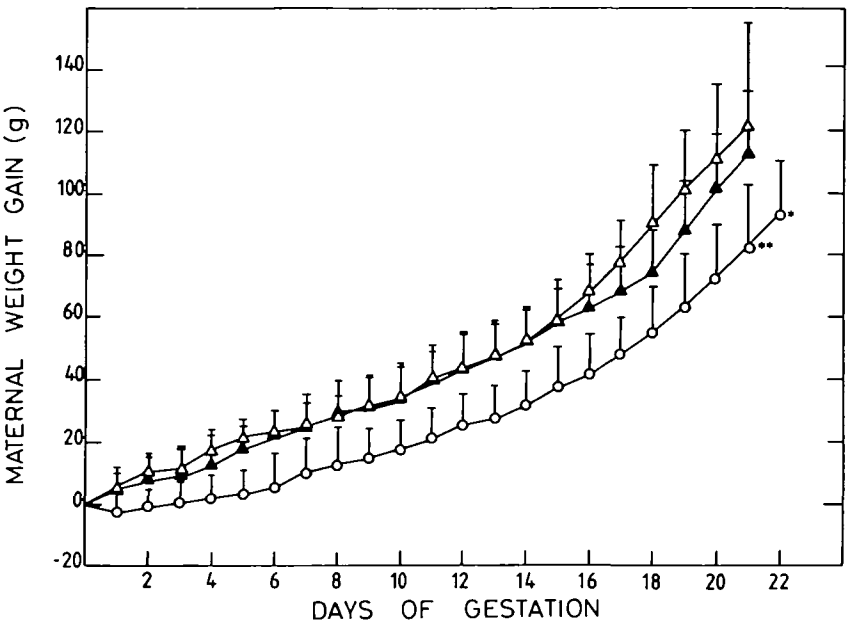

Fig. 1. Maternal weight gain (g) from chow-water (△), pair-fed (▲) and alcohol-treated (○) rats during gestation. Mean ± S.D. for 35–50 pregnant rats/group. Significance of differences from other groups: $^{**}P \leq 0.001$; $^{*}P \leq 0.005$.

Table 3. Effect of chronic ethanol consumption on foetal viability

Gestation time (days)	Groups	Pregnant females	Females with resorption (%)	Total implantation (site/dam)	Total resorption (%)
15	Chow-water	16	6 (37.5)	200 (12.5 ± 3.9)	11 (5.50)
	Pair-fed	14	6 (37.5)	188 (13.5 ± 2.5)	6 (3.19)
	Alcohol-treated	12	10 (83.3)	145 (12.3 ± 2.6)*	26 (17.9)
19	Chow-water	10	1 (10.0)	120 (12.0 ± 3.5)	1 (0.8)
	Pair-fed	10	1 (10.0)	128 (12.6 ± 1.8)	2 (1.5)
	Alcohol-treated	10	6 (60.0)	96 (9.6 ± 3.9)*	9 (10.3)
21	Chow-water	10	2 (20.0)	107 (10.7 ± 4.3)	2 (1.9)
	Pair-fed	14	2 (14.2)	169 (11.7 ± 4.3)	2 (1.1)
	Alcohol-treated	14	7 (50.0)	147 (10.1 ± 3.3)*	9 (6.1)

At 15, 19 and 21 days of gestation, dams were killed and the numbers of implantations and resorptions were recorded. Values in parentheses represent the average ± S.D. Significance of differences with respect to pair-fed group: $*P \leq 0.001$.

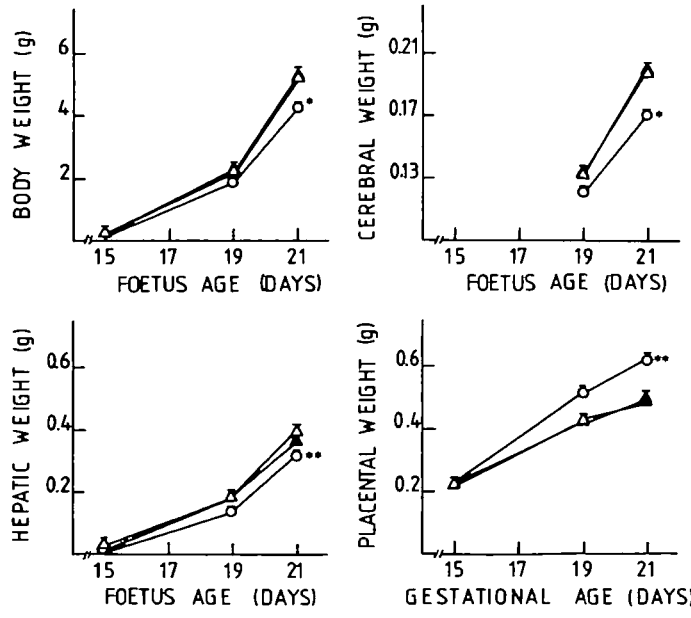

Fig. 2. Effect of maternal ethanol intake on body, brain, hepatic and placental weights. Symbols are as in Fig. 1. Each point represents the average ± S.D. for 12–16 litters from different pregnant rats. Significance of differences from other groups: $**P \leq 0.005$; $*P \leq 0.001$.

Table 4. Analysis of liver and brain of the 21-day-old foetuses

	Alcohol-treated	Pair-fed	Chow–water
Liver			
Protein (mg/g wet wt)	127.0 ± 9.0 (14)	133.0 ± 13 (11)	134.7 ± 4.8 (6)
Glycogen (mg/g wet wt)	54.9 ± 6.2 (8)**	68.0 ± 5.1 (8)	64.4 ± 4.3 (6)
Water content (%)	67.6 ± 2.9 (7)*	72.6 ± 2.2 (8)	75.3 ± 2.1 (8)
Brain			
Protein (mg/g wet wt)	61.6 ± 5.0 (8)**	91.8 ± 11 (10)	82.67 ± 2.7 (6)
DNA (mg/g wet wt)	3.19 ± 0.30 (11)**	3.34 ± 0.36 (11)	3.31 ± 0.4 (7)
(mg/brain)	0.58 ± 0.06 (11)	0.69 ± 0.07 (13)	0.66 ± 0.06 (8)

Significance of differences with respect to the control groups is as follows:
**$P \leqq 0.001$; *$P \leqq 0.005$.
Numbers in parentheses indicate the number of animals used.

Table 5 shows that fewer pregnancies were carried to term in the alcohol-treated dams, i.e. only 78.3% of these rats delivered, in comparison with 91.6% and 93.3%, of the pair-fed and chow-water control rats respectively. This table also shows that ethanol ingestion lengthened the gestation period. Parturition was delayed in alcohol-treated mothers by approx. 1 day, although one delivery was at 25 days. Litter size from alcohol-treated dams was smaller than that from either control group. Intrauterine exposure to alcohol appears to affect foetal weight, because the pups weighed slightly less at birth than their pair-fed counterparts, even with an extra day *in utero*.

That alcohol consumption during gestation has an effect on the postnatal development of body weight is shown in Figs 3 and 4. Nursing pups exposed prenatally to alcohol but maintained with surrogate control mothers during lactation showed significantly less weight gain than either control group. This reduction in body weight persisted during the first two months of life and was more marked in males than in females, but by the 3rd or 4th month the weights approximated those of controls. It may be noteworthy that although female rats recovered their body weight by the fourth month, the weight of their brains remained slightly decreased (pair-fed: 1.73 ± 0.098; alcohol-treated: 1.67 ± 0.053; data from 6–8

Table 5. Effect of prenatal exposure to alcohol on breeding, length of gestation, litter size and body weight of pups at birth

	Groups		
Test	Alcohol-treated	Pair-fed	Chow–water
Positive smears	60	60	60
Successful deliveries (%)	47 (78.3)	55 (91.6)	56 (93.3)
Length of gestation (days)	22.9 ± 0.6*	21.5 ± 0.4	21.7 ± 0.5
Litter size (number)	9.3 ± 2.5*	11.8 ± 2.9	11.9 ± 3.8
Body weight of pups at birth (g)	5.6 ± 0.5	6.0 ± 0.5	6.1 ± 0.5

Values of the last three parameters represent the average ± S.D. of 30–40 pregnant dams.
Significance of difference with respect to control groups: *$P \leqq 0.01$.

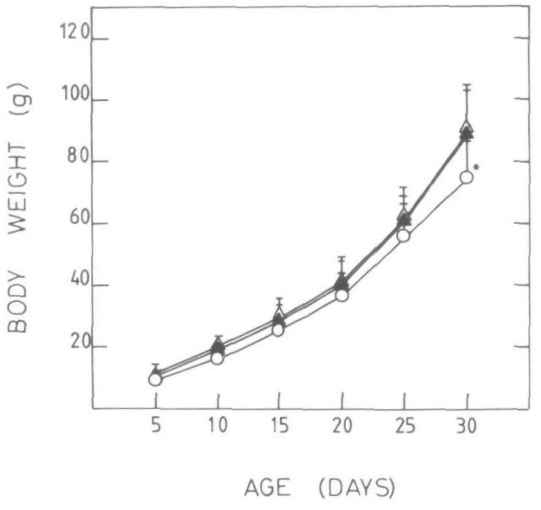

Fig. 3. Effect of prenatal exposure on the body weight of developing rats during lactation. Symbols are as in Fig. 1. Each value represents the average ± S.D. for 5–7 litters. Significance of differences from other groups: $^{*}P \leq 0.001$.

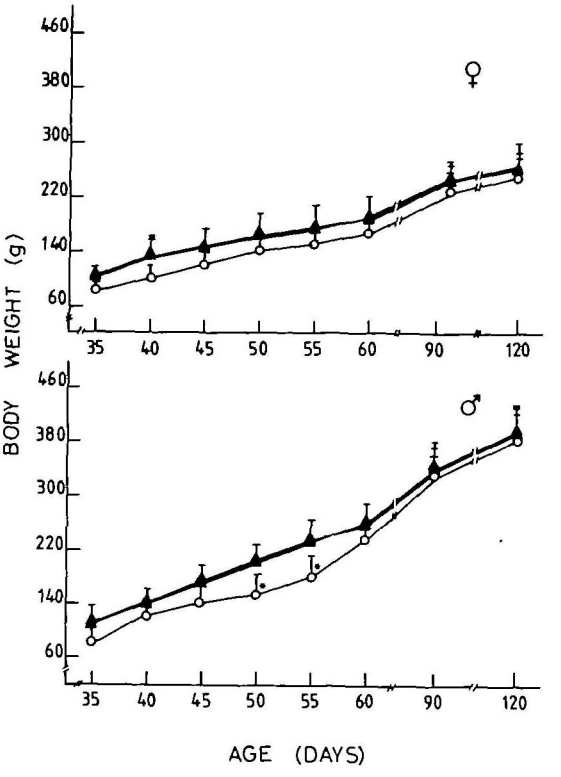

Fig. 4. Effect of prenatal alcohol exposure on the body weight until the 4th month of life. Symbols are as in Fig. 1. Each value represents the average ± S.D. for 4–5 litters. Significance of differences from other groups: $^{*}P \leq 0.05$.

animals/group, mean ± S.D.). Preliminary data indicate that this reduced brain weight occurs also in males.

DISCUSSION

Using an animal model for prenatal exposure to ethanol, we have demonstrated that chronic ethanol consumption during pregnancy is embryotoxic, embryolethal and also toxic to the mother. These adverse effects cannot be attributed to an alcohol-related decrease in food intake, because they were not observed in the pair-fed controls.

Few studies have been reported on the effect of maternal chronic ethanol exposure on foetal development. The females in this study were preadapted to the ethanol-containing diet before mating, thus avoiding the reduced food intake during pregnancy which occurs in the first days on the alcoholic liquid diet and which could be nutritionally harmful for both dam and foetuses. In fact, both caloric and protein intake of our alcohol-treated dams was adequate according to the National Research Council (1972) recommendations for pregnant rats, and the lower weight gain observed in these dams was due to smaller litter size and not to a decrease in food intake.

The increased frequency of resorptions and decreased number of pregnancies carried to term clearly demonstrate the adverse effects of chronic ethanol consumption on foetal viability of rats. These results are in agreement with those of others, when alcohol was administered before and throughout gestation (Henderson *et al.*, 1979; Sorette *et al.*, 1980), but not when the drug was introduced only during pregnancy (Wiener *et al.*, 1981; Weinberg, 1985). It is apparent that alcohol can play an important role in embryolethality. Chronic ethanol intake in humans has also been associated with up to 2- and 3-fold increases, respectively, in the risk of spontaneous abortion and the incidence of

stillbirth (Kline *et al.*, 1980; Sokol *et al.*, 1980). Although immediate causes are unclear, it is known that alcohol interferes with the normal function of the reproductive system: e.g. alcohol alters the oestrous cyclicity and levels of prolactin and luteinising hormone (Sanchis *et al.*, 1985), disrupts the normal migration of sperm into the tubes (Sharma and Chaudhury, 1970), and prevents conception (Chaudhury and Matthews, 1966) and implantation (Tittmar, 1978), etc.

We also found that chronic ethanol intake lengthened (by approx. 1 day) the gestation period, as has been reported by others (Abel *et al.*, 1979). This may be attributed to the inhibitory effect of alcohol on uterine contraction (Fuchs *et al.*, 1967); in fact, alcohol has been used clinically to retard premature labor (Castr *et al.*, 1975). On the day of parturition, when the alcohol-treated dams reduced their food intake, most of them showed low blood ethanol levels (approx. 4.2 mmol/1) during delivery. Blood alcohol levels in their newborn were always higher, as has also been reported in humans (Grüner, 1957; Hanson *et al.*, 1976). These higher levels may be due to the immaturity of the newborns' alcohol-metabolising systems (Idänpään-Heikkilä *et al.*, 1972).

Reports in the literature vary as to the effect of ethanol during pregnancy on weight of the placenta; some have found them unchanged (Leichter and Lee, 1982), whereas others have noted an increase (Wiener *et al.*, 1981), as we have here. The cause(s) for this increase remains unknown; analysis of placentas from alcohol-treated rats has shown only a slight decrease in the protein content and no apparent change in that of water (Sanchis and Guerri, 1985). The hypertrophy of the placenta may be a compensatory mechanism for the decreased blood flow to this organ which occurs during maternal alcohol consumption (Mukherjee and Hodgen, 1982).

The liver, which is one of the organs most damaged by chronic alcohol consumption (Lieber, 1980), is also affected by prenatal exposure (Renau-Piqueras *et al.*, 1985). In the present study, we have found a significant decrease in both glycogen and water in the foetal 'alcohol' livers. A decreased gluconeogenesis has, in fact, been reported in chronic alcoholism (see e.g. Krebs *et al.*, 1969).

The decrease in weight of the foetal brain exposed to ethanol, as well as in its protein and DNA content, implies a decrease in cellularity, which could well be the primary cause of the reduced brain weight observed in foetuses chronically exposed to alcohol. Indeed studies *in vivo* (Rawat, 1975) and *in vitro* (Dreostie *et al.*, 1981) have demonstrated that ethanol alters protein and DNA synthesis. Previous work from this laboratory has shown a reduction in several membrane-bound enzymes, which could also be related to poor neural development in these animals (Guerri *et al.*, 1984; Sanchis *et al.*, 1984).

The reduction in foetal body weight in chronic alcoholism has also been confirmed. The pups from alcohol-treated mothers have a lower birth weight despite an additional day *in utero*; the decreased weight continued during lactation, and was more marked in males than in females. However, as adults, these animals attained body weights similar to the controls, although the brain weight did not recover fully.

Although there have been few descriptions of adolescents or adults born with FAS, mental retardation seems to be maintained, but the deficiencies in weight do not always persist (Streissguth and Clarren, 1984) especially in girls (Clarren, 1985).

Finally we must emphasise that the chronicity of the mother's alcoholism is closely related to the severity of effects in the descendants (Majewski, 1981; Streissguth and Martin, 1983). In the present study, only a few malformations were observed in the progeny. However, in a preliminary study in which female rats were subjected to long-term alcohol treatment (approx. 15 weeks), 60% of the descendants showed apparent fetal malformations (anencephaly, craniofacial features, etc.). The correlation of the stage of the alcohol illness of the mother and the adverse effects on the progeny needs further investigation.

Acknowledgements — We thank Dr S. Grisolía and Dr F. Thompson for their helpful criticism. This research was supported by the Ministerio de Sanidad of Spain, by CAICYT (No. 2108-83) and by a FISS fellowship.

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