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Effects of Prenatal and Postnatal Exposure of Rats to Alcohol: Changes in (Na^+-K^+) ATPase

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GUERRI, C. AND S. GRISOLÍA. *Effects of prenatal and postnatal exposure of rats to alcohol: Changes in (Na^+-K^+) ATPase.* PHARMAC. BIOCHEM. BEHAV. 17(5) 927–932, 1982.—Maternal ethanol consumption produces a reduction in postnatal growth. We have studied especially changes of liver and brain. This reduction is more marked if the alcoholic offspring are maintained with their biological mothers than if they are kept with surrogate mothers. Rats exposed prenatally to alcohol show a marked accumulation of fat in the liver and a significant proliferation of liver endoplasmic reticulum. No change in the postnatal development of liver alcohol (ADH) and acetaldehyde dehydrogenases (ALDH) (high and low K_m) is observed in offspring from alcoholic mothers, with the exception of slightly higher ALDH (low K_m) for the offspring that remain with alcoholic mothers. The postnatal development of the liver (Na^+-K^+) ATPase is also similar in control and alcoholic groups. However, in the case of the enzyme from the brain, a lower ATPase activity is observed in the group derived from alcoholic mothers. Interestingly, at 20 days of postnatal period, an induction of the ATPase (from liver and brain) was observed when the group of offspring from alcoholic mothers were kept on an alcohol diet.

Ethanol Prenatal exposure Postnatal exposure (Na^+-K^+) ATPase

SEVERAL reports suggest that heavy ethanol consumption during pregnancy may be etiologically associated with retarded fetal development and offspring abnormalities in both experimental animals [2,29] and human subjects [16,18].

Ethanol is catabolized in animals via alcohol dehydrogenase (ADH), to acetaldehyde which is then oxidized by acetaldehyde dehydrogenase (ALDH) to acetate. These enzymes as well as many others in liver and brain are known to change during fetal and postnatal development, and experimental evidence indicates that the capacity of young animals to metabolize alcohol is much lower than that of adult animals [21].

Ethanol consumption in the adult rat produces marked morphological alterations in the liver and increases brain (Na^+-K^+)ATPase and liver ALDH [12]. It would be desirable to know if the offspring of alcohol-fed animals show (a) ultrastructural and morphological alterations, (b) if a number of enzymes are affected during development. To that end, we have measured the levels of liver and brain (Na^+-K^+)ATPase, and of alcohol dehydrogenase and aldehyde dehydrogenase from liver. These measurements, together with an electron microscope study of livers and growth curves of offspring of alcoholic mothers kept on alcohol diets as well as offspring of alcoholic mothers kept with surrogate mothers, are presented here.

METHOD

Treatment of Rats

Female Wistar rats weighing between 200 and 250 g were used. All rats were maintained on a 12-hour light day cycle in stainless steel cages. They received the Lieber DeCarli liquid diet containing either 5% (v/v) ethanol or isocalorically balanced with maltose-dextrin for pair-fed controls [19]. A number of female rats were maintained on the ethanol liquid diet for a minimum of 30 days prior to exposure to male rats. Then pairs of a male and a female rat were housed in separate cages. The male animals were removed from the cages of their mates once pregnancy was confirmed. Immediately after birth half of the ethanol-group newborns were left to be fed by the mother kept on the ethanol liquid diet and the other half were assigned to a control surrogate mother that had delivered no more than 24 hr before. The offspring were kept with the mothers until day 20. After this period, all rats were kept on a control liquid diet except when indicated. No significant difference in weight gain of pregnant rats from the ethanol-fed and pair-fed control group was observed at any stage of gestation. For compactness no data is included regarding pups from control mothers raised by non-natural mothers since there were no changes. It is also important to emphasize here the fact that the control liquid diet group was pair-fed both in the prenatal and postnatal periods. We did observe a ~50% increase in neonatal mortality in the ethanol treated group, as illustrated in Table 1.

Enzymatic Measurements

At different times, pups born to control and ethanol-fed mothers were weighed and killed by decapitation. Brains and livers were immediately removed and weighed. The brain

TABLE 1

EFFECTS OF ETHANOL EXPOSURE ON NEONATAL MORTALITY

	Control	Ethanol
Number of pups used	60	185
Pups surviving birth	60	158
Cannibalism on first day postpartum	0	63
Total pups left at day 2	60	95

We used nine control dams and 30 experimental dams for this study.

and half of the liver were homogenized in 9 volumes of cold 0.01 M Tris-HCl (containing 0.15% Na-deoxycholate) with a Super Dispox Tissumizer at full speed, with cooling by immersion in an ice-bath. The (Na^+-K^+)ATPase activity was measured in the homogenate. The assay mixture contained in 1 ml: 100 mM Tris-HCl pH 7.4, 5 mM Tris-ATP, 5 mM $MgCl_2$, 100 mM NaCl, 15 mM KCl and 50–100 μl of the tissue sample. Inorganic phosphate was determined after 10 min incubation at 37°C [8]. The ouabain insensitive Mg^2-ATPase was calculated as the activity measured when Na^+ and K^+ were omitted from the incubation mixture in the presence of 10^{-3} M ouabain. The (Na^+-K^+)ATPase was calculated by difference.

The other half of the liver was homogenized in 9 volumes of cold 0.25 M sucrose with a Super-Dispox Tissumizer at full speed. Part of the homogenate was treated with 0.3% Na-deoxycholate to measure aldehyde dehydrogenase, and another portion was centrifuged at 9000 × g for 10 min in a Sorvall refrigerated centrifuge. The supernatant was assayed for alcohol dehydrogenase activity by the method of Bonnichsen and Brink [7]. Aldehyde dehydrogenase was assayed by the methods of Korivula and Koivusalo [17].

For the electron microscopy study, small liver samples were fixed for 1 hr at 4°C with 2% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 and washed in this buffer. Then the samples were postfixed for 1 hr with 1% osmium tetroxide in a 0.05 M acetate veronal buffer, pH 7.3, dehydrated in acetone and embedded in Epon 812 by standard methods. Ultrathin sections of these blocks were stained with uranyl acetate and lead citrate and finally observed in a Philips EM 300 microscope.

Blood alcohol levels were measured enzymatically [6]. Statistical comparisons were made using Student's t-test.

RESULTS

Postnatal Growth and Development

The effects of in utero exposure to alcohol on postnatal growth is presented in Fig. 1. Since alcohol affects maternal behavior [3] and lactation performance [1], we divided the offspring from alcohol-fed mothers in two groups; those left with their biological mother are called group A and those placed with normal surrogate mothers that had just given birth are called group B. It is apparent from the data shown in Fig. 1 that both groups show a marked inhibition of growth, already significant by day 5, when compared to a pair-fed control group called group C. The marked difference

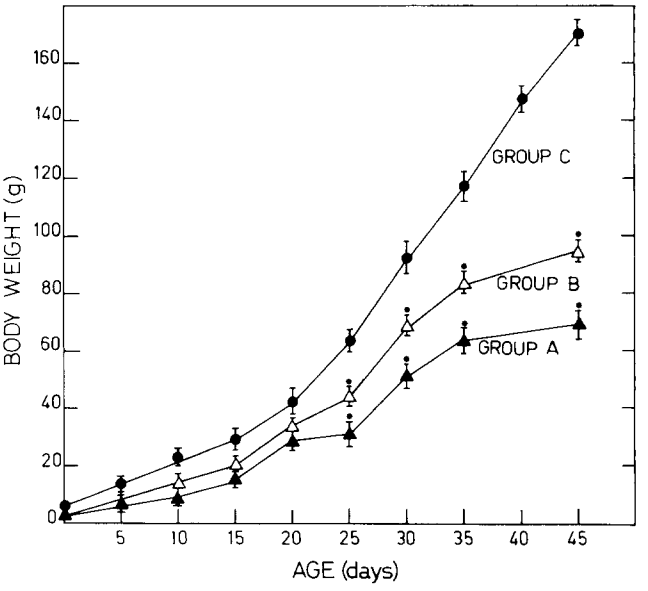

FIG. 1. Development of body weight of offspring from alcoholic mothers, group A (▲) and B (△), and from control group, group C (●), during the postnatal period. Each point represents the mean of 5–7 litters ±S.D. *Significantly different from control group C ($p<0.01$).

between control and experimental groups in weight gain was maintained for the length of this study. There was also less growth in the pups of the group kept with alcoholic mothers.

A similar decrease was noted in the weight gain of organs such as liver (Fig. 2) and brain (Fig. 3). In the latter case, a difference in weight between control and experimental groups was already noticeable at birth and it remained essentially without variation after day 20 (Fig. 3). Again, the tissues of animals kept with alcoholic mothers grew less. However, if we express the data as relative weights (organ weight/body weight) from the time of birth on, the percentage of weight of liver and brain was larger than for the controls (Table 2).

Ultrastructural Liver Study

The normal liver morphology of offspring from control animals is shown in Fig. 4A. Liver from rats prenatally exposed to alcohol shows a marked accumulation of fat and a significant proliferation of the endoplasmic reticulum. We did not find any alterations in the mitochondrial structure (Fig. 4B).

Developmental Changes in Alcohol Dehydrogenase and Aldehyde Dehydrogenase Activities

The developmental pattern of ADH activity is shown in Fig. 5. In control rats there is a linear increase in the activity with age, reaching adult levels at approximately 20 days and a peak of higher activity at about 25 days as reported by others [21]. The significance of such a peak is not known, nor why the offspring of alcoholic mothers show a small or no peak (groups A and B, respectively).

The increase of high K_m acetaldehyde dehydrogenase ac-

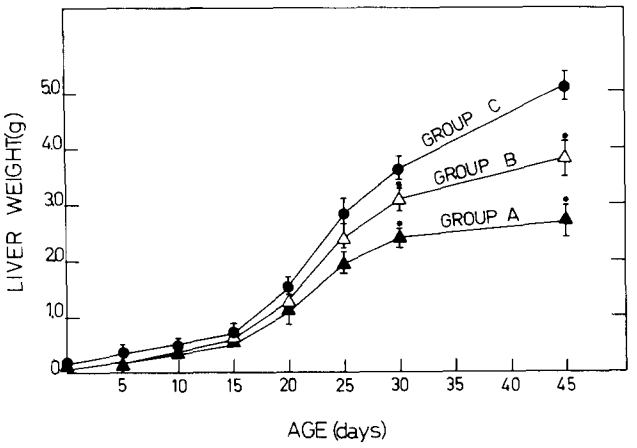

FIG. 2. Development of liver weight of offspring from alcoholic mothers, group A (▲) and B (△), and from control group, group C (●), during the postnatal period. Each point represents the mean of 5–7 litters ±S.D. *Significantly different from control group C ($p \leq 0.01$).

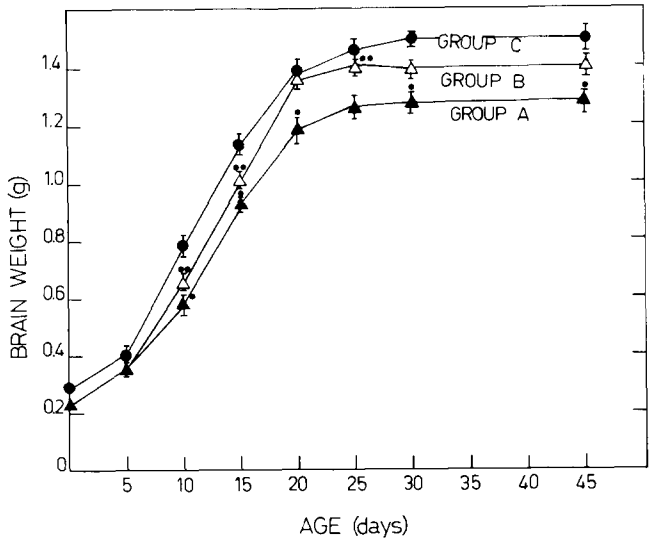

FIG. 3. Development of brain weight of offspring from alcoholic mothers, group A (▲) and B (△), and from control group, group C (●), during the postnatal period. Each point represent the mean of 5–7 litters ±S.D. *Significantly different from control group C ($p \leq 0.01$). **Significantly different from control group C ($p \leq 0.05$).

tivity with age is presented in Fig. 6. The activity from control rats increases, reaching adult levels at 25 days. A similar pattern was found in the offspring of alcoholic rats. A small decrease in the activity levels was seen in the offspring from alcohol-fed rats when kept with their biological mothers.

Interestingly, as illustrated in Fig. 7, the development of the low K_m acetaldehyde dehydrogenase which seems to be responsible for at least 80–90% of the oxidation of acetaldehyde during ethanol metabolism [28] is higher for group A than for groups B and C.

TABLE 2

EFFECT OF PRENATAL AND POSTNATAL ALCOHOL CONSUMPTION ON RELATIVE WEIGHTS ORGAN WEIGHT/BODY WEIGHT × 100

Days	Liver Groups			Brain		
	A	B	C	A	B	C
0	25	25	37.5	5	5	5
5	30	18.75	21.43	7	4.2	2.9
10	27.8	25	23.8	6.4	4.7	3.8
15	32	27.5	24.38	6.6	5	3.9
20	39.3	38.2	35.7	4.2	2.9	3.3
25	58	52.3	46	4.1	3.2	2.3
30	47	45	40	2.5	2	1.6
45	38.6	40.6	30.4	1.8	1.46	0.91

Group A represents alcoholic pups kept with alcoholic mothers.
Group B represents alcoholic pups kept with surrogate control mothers.
Group C represents pair-fed controls.

Developmental Changes in (Na^+-K^+)ATPase of Liver and Brain

The increase in liver (Na^+-K^+)ATPase with age is shown in Fig. 8. Since, as shown, the development of the enzyme activity was similar in control and in the experimental groups, twenty days after birth we separated the offspring from their mothers and put half of the pups which had been kept with the alcoholic mothers on an isocaloric liquid diet (group 1) and kept the other half on an alcohol liquid diet (group 2). We then followed the activity level until day 45. As shown, we observed an increase in the (Na^+-K^+)ATPase activity in the group maintained on an alcoholic diet with respect to the group maintained on the control diets.

Figure 9 shows that with the (Na^+-K^+)ATPase from brain the development of activity in the offspring from alcoholic mothers was lower than that in the offspring from control rats. However, when the group of alcoholic offspring kept with their alcoholic mothers for 20 days was changed to an alcohol diet, a moderate increase of the enzyme activity was observed, as expected from previous work [12].

DISCUSSION

We show in this paper, in agreement with other reports [2,29], that maternal ethanol consumption by the rat causes a marked postnatal inhibition of growth in their offspring, with decreased brain weight and reduced postnatal survival. Our findings also show that alcohol consumption of the mothers during the lactation period further reduces growth of the brain, liver and total weight. The effects on liver and brain may be due to decrease in hepatic and brain protein synthesis [22,23]. Also, it has been found recently that the most significant inhibition of protein synthesis was in the developing brain of suckling neonates whose mothers drank ethanol immediately after birth and through the entire first week of the postnatal period [27]. However, we have seen that the relative weights of the experimental groups are bigger than the C groups. We do not know why this alteration occurs; it may be due to hormonal changes.

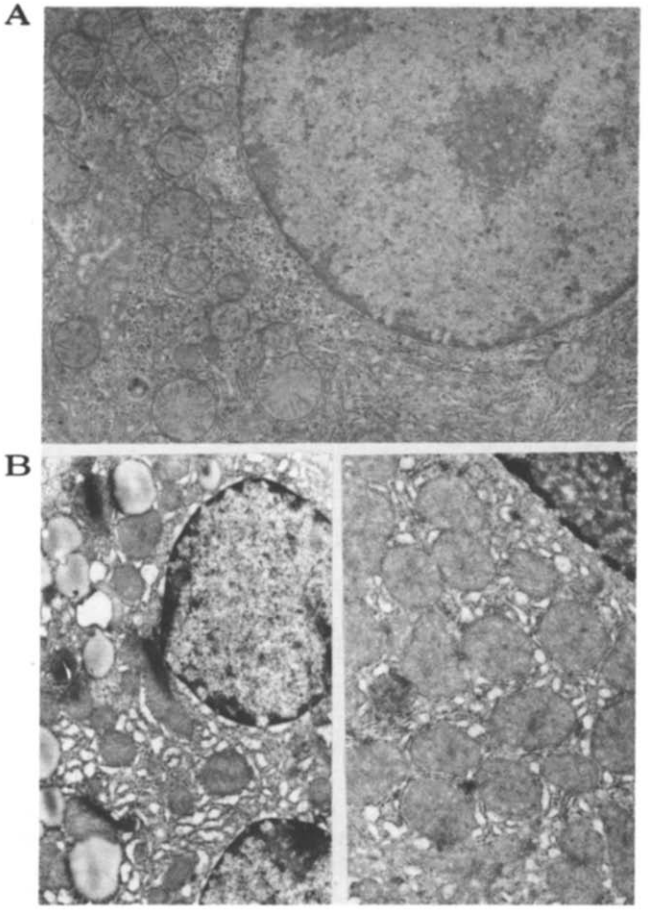

FIG. 4. Electromicrograph from liver of offspring from control animals (1 day) (A) and from liver of rats prenatally exposed to alcohol (B).

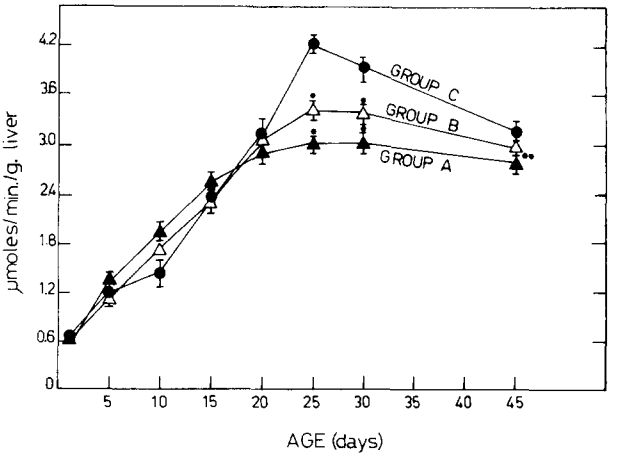

FIG. 5. Developmental changes of liver alcohol dehydrogenase from offspring of alcoholic mothers, group A (▲) and B (△), and from control animals, group C (●). Each point represents the mean of 5–7 different values of different animals ±S.D. *Significantly different from control group C ($p \leq 0.01$). **Significantly different from control group C ($p \leq 0.05$).

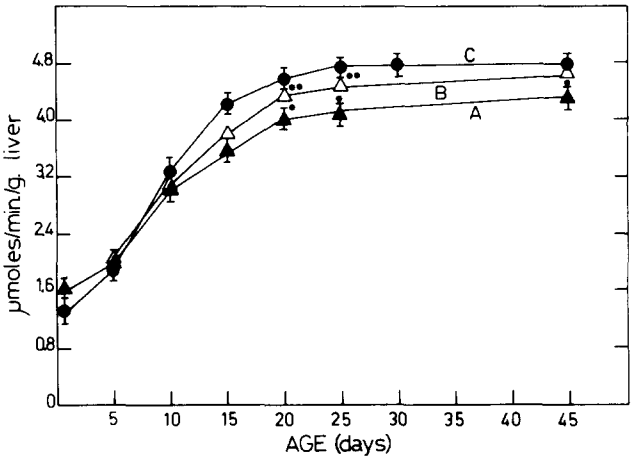

FIG. 6. Developmental changes of liver aldehyde dehydrogenase (high Km) from offspring of alcoholic mothers, group A (▲) and B (△), and from cmntrol animals, group C (●). Each point represents the mean of 5–7 different values of different animals ±S.D. *Significantly different from control group C ($p \leq 0.01$). **Significantly different from control group C ($p \leq 0.05$).

Alterations in liver morphology such as changes in the configuration of mitochondria, development of the endoplasmic reticulum, etc. have been reported in humans and in animals after chronic ethanol intake [19,25]. In fact, histological abnormalities of liver have been reported in three patients with fetal alcohol syndrome [13] and an increase in hepatic lipids and triglyceride content has been shown in offspring of alcoholic rats [24]. We have now found a marked accumulation of fat and a significant proliferation of the endoplasmic reticulum in livers of rats prenatally exposed to alcohol. However, we did not find any alteration in the mitochondrial structure.

No changes in the development of ADH and ALDH (high K_m) of liver in offspring from control and alcohol rats was found except in group A, which showed a slight decrease in the levels of these enzymes. This decrease may be due to acetaldehyde inhibition of ADH and ALDH [10,11] and/or alteration of the protein intake in alcoholic mothers. However, this group (group A) shows an increase in the levels of (low K_m) ALDH, possibly due to a physiological adaptation since this enzyme has been postulated to be responsible for at least 80–90% of the oxidation of acetaldehyde during ethanol metabolism [28].

On the other hand, it is known that the major changes in enzymatic activity occur during the maturation of the brain and especially during one of the critical periods of development (from day 18 through day 40). This period is the time of major development of the glial and myelin structures of the nervous system with formation of synaptic connections [9]. Other studies have shown [20,26] that neonatal thydroidectomy and undernutrition produce a marked decrease in the activity of some membrane-bound enzymes.

The (Na^+-K^+)ATPase is known to be related to the structure of the cell membrane, and thus to transport mechanisms. Also, this enzyme is particularly active in brain re-

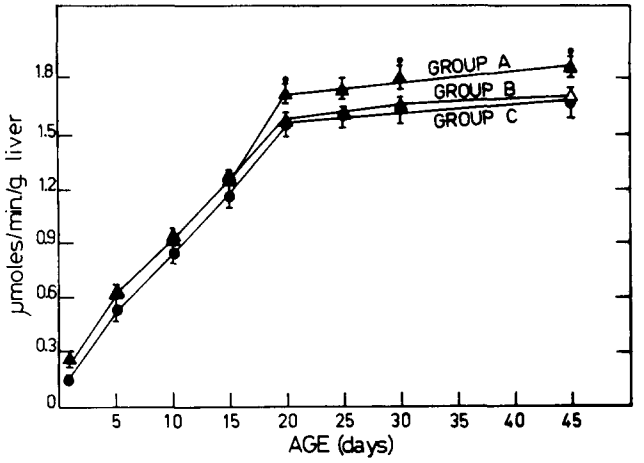

FIG. 7. Developmental changes of liver aldehyde dehydrogenase (low Km) from offspring of alcoholic mothers, group A (▲) and B (△), and from control animals, group C (●). Each point represents the mean of 5–7 different values ±S.D. *Significantly different from control group C ($p \leq 0.01$).

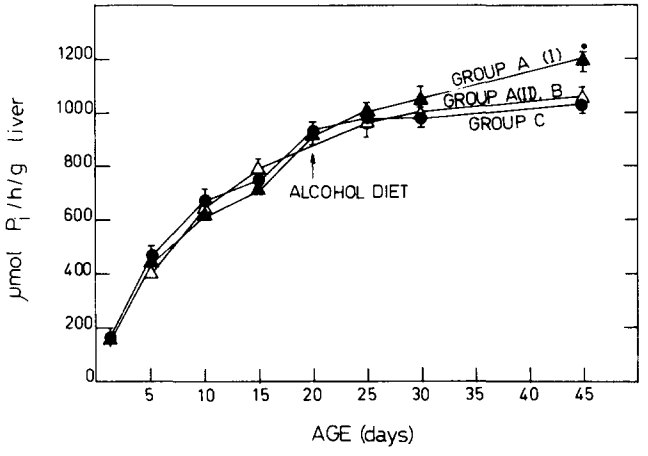

FIG. 8. Developmental changes of liver (Na^+-K^+)ATPase from offspring of alcoholic mothers, group A (▲) and B (△, and from control animals, group C (●). After day 20 of postnatal period the rats from group A were fed with an alcohol liquid diet until 45 days. Each point represents the mean of 5–7 different values of different animals ±S.D. *Significantly different from control group C ($p \leq 0.01$).

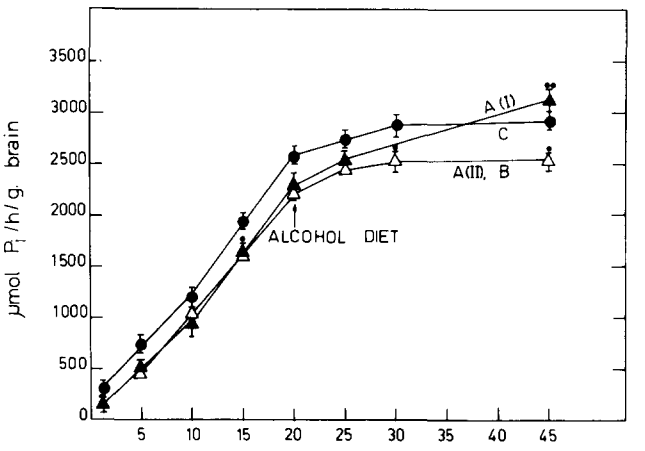

FIG. 9. Developmental changes in brain (Na^+-K^+)ATPase from offspring of alcoholic mothers, groups A (▲) and B (△), and from control animals, group C (●). After day 20 of postnatal period the rats from group A were fed with an alcohol liquid diet until day 45. Each point represents the mean of 5–7 different values of different animals ±S.D. *Significant different from control group C ($p \leq 0.01$). **Significantly different from control group C ($p \leq 0.05$).

gions with high concentrations of synaptic membranes, such as gray matter, and in synaptosomes. As shown above, we found a lower activity in brains of offspring from alcoholic mothers. This finding may be due to a combination of several causes: (1) a general metabolic impairment of brain; neuronal and brain disturbances have been reported in the fetal alcohol syndrome [15]; (2) a direct inhibition of this enzyme by ethanol [14]; and (3) a greater sensitivity and thus alteration in brain membrane and/or enzyme [21] conformation due to a change in lipid configuration or composition. The first and second causes seem more probable since the development of the (Na^+-K^+)ATPase from liver does not change.

Interestingly, the level of brain (Na^+-K^+)ATPase increases when normal rats are given alcohol diet, as reported by us and others [4,12]. The reason for this remains obscure; Bernstein *et al.* [5] speculate that it might be due to a compensatory mechanism to overcome the inhibition of this enzyme by ethanol or, secondarily, to ethanol-induced hormonal changes.

The findings presented here demonstrate clearly a gross inhibition of growth and developmental derangements in neonatal animals exposed to ethanol. We have also demonstrated changes in the development of some liver and brain enzymes. Additional experiments are needed to clarify whether or not there are alterations or changes in membrane-bound enzymes per se or whether the changes are due to alterations in membrane composition in rats prenatally exposed to alcohol.

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