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Liver Alcohol Dehydrogenase Activity and Ethanol Levels During Chronic Ethanol Intake in Pregnant Rats and Their Offspring

CARMEN TRAVÉS, LAURA CAMPS AND DOLORES LÓPEZ-TEJERO[1]

Unitat de Bioquímica i Biologia Molecular B, Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08071 Barcelona, Spain

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TRAVÉS, C., L. CAMPS AND D. LÓPEZ-TEJERO. *Liver alcohol dehydrogenase activity and ethanol levels during chronic ethanol intake in pregnant rats and their offspring.* PHARMACOL BIOCHEM BEHAV 52(1) 93-99, 1995.—The effects of chronic alcohol intake on the ethanol levels in body fluids (blood, amniotic fluid, and fetal intragastric content), hepatic alcohol dehydrogenase (ADH) activity, isoenzyme distribution, and hepatic zinc levels were studied in pregnant rats at term (19 and 21 days), in their offspring at fetal, perinatal, and weaned stages, and in adult virgin rats. Three experimental groups were studied: 1) the alcohol group received ethanol in drinking water (from 10% to 25% over 2 months), 2) the fibre diet group was undernourished on a hypocaloric diet, to assess the effects of malnutrition associated with chronic alcohol intake, and 3) the control group received no alcohol and normal diet. A gradient of increasing ethanol concentrations was found in fetal blood, amniotic fluid, and fetal intragastric contents with respect to maternal blood. A decrease in ADH activity was found in alcohol-consuming pregnant rats compared to controls. This was related neither to liver ADH isoenzyme distribution nor to changes in hepatic zinc levels. Chronic alcohol consumption in pregnant rats produced high ethanol accumulation in fetal fluids and changes in the liver ADH activity depending on the physiological situation (pregnancy, development, virgin state).

Alcohol dehydrogenase (ADH) Ethanol Pregnancy Liver Zinc (Zn) Rat

ADVERSE effects on the fetus and newborn after alcohol exposure in utero (fetal alcohol effects and fetal alcohol syndrome) (1,49), ranging from morphological defects to growth retardation and central nervous system dysfunctions (34, 43,44), have been reported.

Many variables determine the alterations observed in offspring following gestational ethanol intake (10,18). Alcohol per se may be toxic to the fetus, because ethanol crosses the placenta (16,20) and rapidly establishes an equilibrium between maternal and fetal fluids (20,26). The principal site of ethanol elimination from the body is the liver, where ethanol is metabolized to acetaldehyde mainly by cytosolic alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase, EC 1.1.1.1, ADH) (28). In the rat, ADH activity in liver (mainly associated with the isoenzyme ADH-3) accounts for 90% of total ADH (5). Moreover, fetal liver ADH is detectable at low levels at the end of pregnancy (27,42,46,48) and none of the ADH that metabolizes ethanol has been found in placenta (40, 46,48).

A range of chronic alcohol models have focused on the late gestational period, when fetal growth and nutritional demands on the mother are greatest. The malnutrition associated with chronic ethanol intake (30) must be taken into account when studying the fetal impairment caused by ethanol exposure (51). The chronic model that we use has been described elsewhere (51,52). Ethanol (10–25% w/v) is administered to the mother via the drinking water, and a variety of alterations are subsequently observed during prenatal and postnatal development of the offspring (32–34,37).

The effects of chronic ethanol intake in rats on liver ADH activity are controversial because of differences in assay conditions (8,19,29,36,56). The divergent results indicate the need for further studies to relate enzyme activity to the circulating ethanol levels, especially in pregnant animals and their off-

[1] To whom requests for reprints should be addressed.

spring. The aim of this study was to determine the ethanol levels in various fluids (blood, amniotic fluid, and intragastric fetal contents) and hepatic ADH activity. Ethanol-treated pregnant rats, their offspring and virgin rats were studied. Parallel determinations of liver ADH isoenzyme distribution and hepatic zinc content were performed to evaluate possible relationships with variations in the ADH activity.

METHOD

Animals and Chronic Ethanol Treatment

Adult female Wistar rats from our own colony with an initial body weight of 160 ± 5 g were used. They were housed in an environment in which temperature (23°C), humidity (75%), and light cycle (on from 0800 to 2000 h) were controlled. Groups of three animals were kept in plastic wire-topped cages until the experiments began, and they were fed solid rodent chow and tap water ad lib (Panlab, Barcelona, Spain).

Animals were divided into the following experimental groups, as described elsewhere (51): alcohol-treated rats (A) given ad lib 10% ethanol (w/v) in drinking water for 1 week, 15% during the second, 20% during the third, and 25% during the fourth week (pregestational period). At the end of the fourth week one nontreated male was put into each cage during the 12-h dark periods until spermatozoa appeared in vaginal smears (day 0 of gestation). Pregnant rats were maintained on 25% ethanol (w/v) in the drinking water until the end of the gestational period (3 weeks). Female rats that had not mated after 5 days were used to form the group of virgin rats, which drank 25% ethanol solution for the following 3 weeks. Control rats (C) receiving no alcohol were given drinking water ad lib, but were otherwise handled like the alcohol group. The fibre diet group (FD) received a diet diluted to 50% with pure cellulose ad lib (Panlab) but otherwise these animals were handled like the control group. The fibre diet group was used as control for the malnutrition associated with chronic ethanol intake. Total caloric intake in fibre diet animals decreased to 75–80% of age-matched control and alcohol groups throughout the chronic treatments (pregestational and gestational periods) (51). This percentage of calories was similar to the caloric intake from solid food in the alcohol-treated animals (51). Furthermore, the growth rate in fibre diet group (undernourished control group) was similar to that of the alcohol-treated rats (51).

All the animals were killed by decapitation at the same time of day (around 1000 h) to avoid circadian variations. Samples were taken from virgin rats (V), pregnant rats (0P, 19P, and 21P) and their fetuses (19F and 21F) (removed by cesarian section). Samples were also taken from two groups of pups born by natural delivery: one on the day of birth (B) and the other at weaning (W) (30 days later). 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. Livers of adults, fetuses, and pups were dissected out immediately, weighed, frozen in liquid nitrogen, and stored at −30°C for ADH, protein, and zinc assays. Fresh tissue was used for starch gel electrophoresis of ADH isoenzymes.

Blood (from the neck) and fetal fluids (amniotic and intragastric) were collected for ethanol determination only from the alcohol group.

Ethanol Determination

Ethanol concentrations were determined in fluid samples quickly collected into cold heparinized receptacles for immediate deproteinization with 3% (v/v) perchloric acid in the proportion 1 : 4 (v/v). Samples were centrifuged at 12,000 × *g* for 2 min at 4°C and the supernatants were used, on the same day, for ethanol determination with a spectrophotometric assay at 340 nm (15) using a Shimazu UV-160 A spectrophotometer. Results are expressed as μg of ethanol per ml.

Liver Processing

Livers were thawed, cut into small pieces, and homogenized in ice-cold 10 mM Tris-HCl, pH 8.8, 0.5 mM dithiothreitol (25), using a 1 : 5 (w/v) dilution in adults and 30-day-old pups, and 1 : 1 (w/v) in fetuses and newborn pups, with a glass-to-glass homogenizer in an ice bath. The homogenates were centrifuged at 27,000 × *g* for 1 h at 4°C (25). Diluted supernatants were used for ADH determinations and starch gel electrophoresis; no changes were observed in the activity of fresh or frozen tissue after storage at −30°C.

Alcohol Dehydrogenase Assay

ADH activity was determined by measuring the increase in absorbance at 340 nm in a Shimazu UV-160 A spectrophotometer. The liver ADH assay was performed in a medium containing 0.1 M glycine/NaOH, pH 10.0, with 2.4 mM NAD^+. The assay was performed in a water bath at 37°C for 4 min. The reaction was initiated by adding ethanol to a final concentration of 15 mM and stopped with a specific inhibitor of ADH (pyrazole) at final concentration of 10 mM (25). Parallel blanks were used for each liver sample, in which the inhibitor was added before the ethanol. Studies of the linear reaction were performed and during the time assayed the reaction was completely linear. Protein concentration was determined in the homogenates following Lowry (35), using bovine serum albumin as standard. Specific ADH activity was expressed as units (U) per g of liver protein. One unit of enzymatic activity was considered to correspond to 1 μmol of NADH formed per minute.

Starch Gel Electrophoresis

Electrophoresis on starch gels was performed as previously indicated (41), using 0.1 M 2-buten-1-ol as substrate for the activity staining. Liver supernatants (10 μl) were loaded into the gel.

Zinc Determination

Liver homogenates were diluted with a solution of $HClO_4$: H_2O_2 (1 : 1) at the proportion 1 : 2 (v/v) and kept in darkness overnight. The next day the samples were put into a water bath at 80°C for 2 h and kept in darkness for a second night at room temperature. Finally, double distilled water was added to the samples in the proportion 1 : 6 (v/v). Zinc was determined by atomic absorption spectroscopy (Philips PV9200X Atomic Absorption Spectrometer) using a pure zinc solution (1 g/l, Viñas Laboratory, Barcelona, Spain) as standard. The results are expressed as μg zinc per g liver.

Statistical Analysis

Results are presented as means ± SEM of five to eight animals. Results were statistically tested using Student's *t*-test and analysis of variance (ANOVA) for two factors by Statgraphics Version 5.0 (Statistical Graphics Corp.). The two factors defined were "diet" and "physiological state." The diet factor was defined as control (C), alcohol (A), and fibre diet (FD) treatments. The physiological state factor was defined in

TABLE 1

DAILY ENERGY INTAKE IN PREGNANT AND VIRGIN RATS

	kcal/100 g Body Weight/Day		
	C Total	A Total (derived from ethanol)	FD Total
0P	21.80 ± 1.15	21.47 ± 0.88* (5.74 ± 0.24)	18.00 ± 0.40
19P, 21P	23.65 ± 0.72	23.12 ± 1.95† (8.37 ± 0.31)	18.50 ± 0.60
V	20.10 ± 0.60	21.40 ± 1.50† (8.80 ± 0.60)	17.50 ± 0.52

0P, 19P, and 21P are days of pregnancy and V virgin rats from control (C), alcohol (A), and fiber diet (FD) groups. Each value represents the mean of five to eight animals ± SEM.

Student's *t*-test: *A vs. FD, $p < 0.01$ and †A vs. FD, $p < 0.05$.

adults as origin of gestation (0P), end of gestation (19P, 21P), and virginity (V) and in offspring as fetus (19F, 21F), pups at birth (B), and weaned pups (W). Differences were considered significant at $p < 0.05$. Multiple range analyses for each paramenter by each factor were performed for the interpretations of ANOVA results.

RESULTS

In both virgin and pregnant animals, the alcohol group and the fibre diet group were, respectively, isocaloric and hypocaloric compared to normal diet control group (Table 1).

Ethanol-treated pregnant rats at term and virgin rats drinking 25% alcohol solution consumed an average of 12 ± 3 g ethanol/kg b.wt./day. At the end of the chronic treatment (2 months), the ethanol intake represented between 36% and 40% of the total kcal ingested (Table 1). The fibre diet group (as undernourished control group) showed significant reduction (20–25%) of the total energy ingested with respect to the normal-fed control and alcohol groups.

The last gestational period (19P and 21P) produced a significant increase in maternal body weight, liver weight, liver protein, and hepatic zinc levels (Table 2). Chronic ethanol intake resulted in a significant reduction in body and liver weight, a significant increase in liver protein content, and no modification in hepatic zinc levels compared to control groups (Table 2).

Chronic ethanol intake during late pregnancy resulted in a significant reduction in body and liver weight in offspring, when compared to controls (Table 3). However, the significant differences in liver proteins and zinc were due to the fibre diet intake during gestation (Table 3).

The blood ethanol levels in mother and virgin animals were around 460 μg/ml (Fig. 1). Higher levels than those of maternal blood were found in fetal blood. At the same time ethanol seemed to accumulate in the amniotic and intragastric fluid in fetus (Fig. 1).

Liver ADH specific activity showed a significant decrease during late gestation (19P and 21P) compared with virgin (V) and with the origin of gestation (0P) (Fig. 2). Chronic ethanol intake produced a significant fall in liver ADH activity, especially during late gestation (Fig. 2).

Fetal liver ADH activity was absent on the 19th day of pregnancy but was detected at the end of gestation (21 days); at birth the activity was approximately 20% that of adults (virgin rats) and weaned pups reached the adult levels (Fig. 2). Chronic ethanol treatment in pregnant rats did not lead to changes in liver ADH activity in their offspring (Fig. 2).

The starch gel electrophoresis of liver homogenates (Fig. 3) demonstrated the existence of two different ADH isoenzyme types in pregnant and virgin rats, the minority anodic isoenzyme (ADH-2) and the main cathodic isoenzyme (ADH-3). The specific staining showed different forms of ADH-3 that were similar in both control and alcohol groups (Fig. 3). Fetal liver homogenates showed the existence of one ADH isoenzyme, ADH-3, with only one cathodic form; similar results were found in alcohol and control groups (Fig. 3).

TABLE 2

BODY WEIGHT AND LIVER PARAMETERS IN PREGNANT AND VIRGIN RATS

	Group	Body Weight (g)	Liver Weight (g)	Liver Proteins (mg/100 mg)	Liver Zinc (μg/g)
0P	C	221.43 ± 11.65	8.40 ± 0.24	16.75 ± 1.13	30.71 ± 0.96
	A	201.43 ± 8.31	6.78 ± 0.39	18.09 ± 0.43	31.51 ± 0.98
	FD	235.71 ± 4.63	8.79 ± 0.16	17.23 ± 0.52	30.51 ± 1.25
19P	C	332.40 ± 5.15	13.92 ± 0.37	24.50 ± 1.23	38.03 ± 3.02
	A	268.12 ± 6.57	10.32 ± 0.50	24.07 ± 0.57	33.67 ± 0.84
	FD	328.83 ± 8.93	13.25 ± 0.86	24.18 ± 0.64	36.05 ± 1.25
21P	C	362.43 ± 10.54	11.60 ± 0.25	21.95 ± 0.79	33.03 ± 1.04
	A	279.37 ± 10.01	9.46 ± 0.43	22.10 ± 0.58	29.83 ± 1.03
	FD	351.43 ± 10.13	12.35 ± 0.59	19.82 ± 0.36	34.01 ± 1.78
V	C	267.62 ± 8.40	9.26 ± 0.36	18.36 ± 0.44	29.57 ± 1.03
	A	243.00 ± 17.78	7.09 ± 0.87	20.43 ± 0.77	31.01 ± 0.68
	FD	250.00 ± 2.72	9.08 ± 0.41	17.03 ± 0.61	30.38 ± 0.76
ANOVA					
Diet × physiological state		$F = 3.76, p < 0.008$	NS	NS	NS
Diet		$F = 16.04, p < 0.001$	$F = 22.13, p < 0.001$	$F = 6.89, p < 0.002$	NS
Physiological state		$F = 144.75, p < 0.001$	$F = 90.00, p < 0.001$	$F = 68.77, p < 0.001$	$F = 11.77, p < 0.001$

0P, 19P, and 21P are days of pregnancy and V virgin rats from control (C), alcohol (A), and fiber diet (FD) groups. Each value represents the mean of five to eight animals ± SEM. Significant $p < 0.05$, NS not significant.

TABLE 3
BODY WEIGHT AND LIVER PARAMETERS IN RAT OFFSPRING

	Group	Body Weight (g)	Liver Weight (g)	Liver Proteins (mg/100 mg)	Liver Zinc (μg/g)
19F	C	1.97 ± 0.19	0.16 ± 0.02	14.97 ± 0.44	49.82 ± 2.67
	A	1.83 ± 0.05	0.14 ± 0.00	14.59 ± 0.25	52.43 ± 1.07
	FD	2.19 ± 0.04	0.17 ± 0.01	14.51 ± 0.44	50.97 ± 4.59
21F	C	5.20 ± 0.16	0.39 ± 0.02	12.90 ± 0.79	79.56 ± 2.59
	A	3.92 ± 0.22	0.26 ± 0.02	13.05 ± 0.47	78.24 ± 3.20
	FD	5.00 ± 0.11	0.38 ± 0.01	12.10 ± 0.76	69.19 ± 2.75
B	C	6.35 ± 0.10	0.32 ± 0.01	14.80 ± 0.73	114.96 ± 6.12
	A	5.04 ± 0.34	0.21 ± 0.02	13.56 ± 0.69	94.75 ± 5.83
	FD	5.59 ± 0.12	0.31 ± 0.02	13.00 ± 0.47	99.57 ± 6.44
W	C	105.84 ± 7.00	4.06 ± 0.68	20.60 ± 0.94	44.17 ± 2.33
	A	92.63 ± 6.74	3.56 ± 0.33	18.58 ± 1.56	42.86 ± 2.28
	FD	103.48 ± 4.63	4.12 ± 0.34	19.00 ± 0.50	39.24 ± 2.24
ANOVA					
Diet × physiological state		NS	NS	NS	NS
Diet		NS	NS	$F = 5.38, p < 0.008$	$F = 5.84, p < 0.006$
Physiological state		$F = 1104.46, p < 0.001$	$F = 495.05, p < 0.001$	$F = 102.32, p < 0.001$	$F = 52.73, p < 0.001$

19F and 21F are days of fetal age, B pups at birth, and W weaned pups from control (C), alcohol (A), and fiber diet (FD) pregnant rats. Each value represents the mean of five to eight animals (W) or litters (19F, 21F, and B) ± SEM. Significant $p < 0.05$, NS not significant.

DISCUSSION

The present model of chronic ethanol drinking in the rat during pregnancy reveals considerable prenatal and postnatal effects in offspring and mothers (32-34,51,52), the most evident being the reduction in body, brain, and liver weight. These alterations were also reported by other authors using different models of chronic alcohol ingestion (2,14,18, 24,36,46,53).

The effect of chronic alcohol drinking on body weight was more pronounced during gestation than in virgin rats (Table 2), although the caloric intake and the quantity of ethanol consumed (12 g/kg b.wt./day) was similar in both (Table 1), as reported in previous studies (51,52). It is evident that nutritional requirements during pregnancy are greater and there may be a combination of the effects of ethanol intake and the malnutrition associated with this intake. Previous studies establish a careful control of nutrition in alcohol-consuming rats (51). This is supported by the fact that throughout the chronic treatment in the fibre diet group (undernourished control group) similar growth curves were obtained, and the number of pups per litter was the same as in the alcohol group.

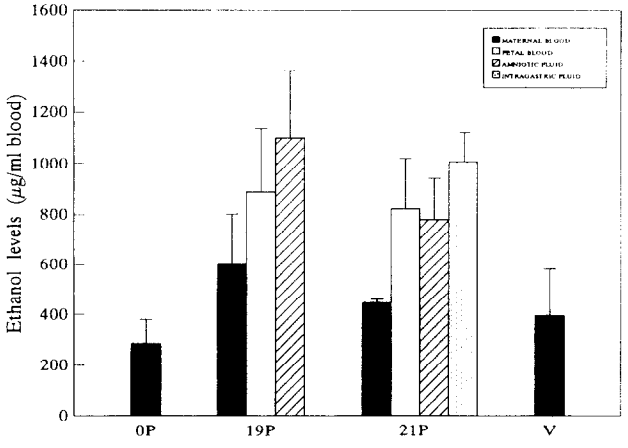

FIG. 1. Ethanol levels in maternal blood and fetal fluids in chronic alcohol group. 0P, 19P, and 21P—days of pregnancy, V—virgin rats. Each bar represents the mean ± SEM of five to eight values from different animals or litters.

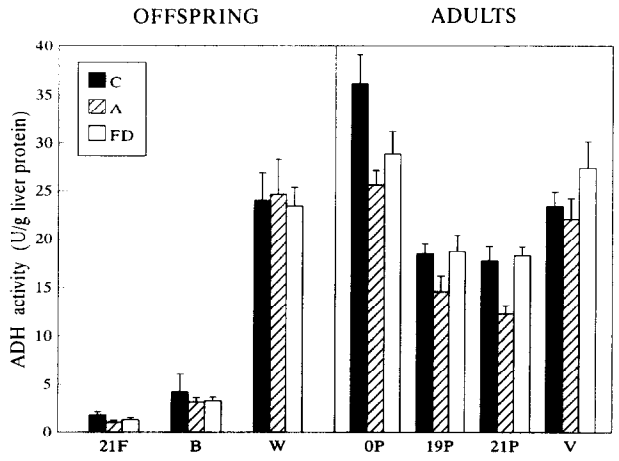

FIG. 2. Specific alcohol dehydrogenase activity of liver. Adults: pregnant (0P, 19P, and 21P days of pregnancy) and virgin (V) rats. Offspring: 21 days of fetal age (21F), birth (B), and weaned pups (W). Control (C), alcohol (A), and fibre diet (FD) groups. Each bar represents the mean ± SEM of five to eight values from different animals. ANOVA offspring: diet × physiological state, NS; diet, NS; physiological state, $F = 275.886$, $p < 0.001$. ANOVA adults: $F = 3.563$, $p < 0.010$; diet, $F = 11.216$, $p < 0.001$; physiological state, $F = 76.877$, $p < 0.001$.

Besides, there were fewer sings of stress than what is found with the use of other types of nutritional control (pair-fed) (51). The wide dispersion of weights obtained in alcohol-treated virgin rats demonstrates the great variability of the effects of chronic ethanol intake. In the fibre diet group such variation in the body weight in virgin rats was not observed, but mean values were similar to the alcohol group (51).

We found an increasing gradient of ethanol concentration in fetal blood, amniotic fluid, and intragastric fetal content with respect to maternal blood, which demonstrates that ethanol crosses the placental barrier (16,20) and is distributed in the maternal and fetal compartments. The accumulation of ethanol observed in fetal fluids could be related with various phenomena. First, the fetus has a low capacity to eliminate ethanol, which is thus returned to the mother for oxidation (9,20,39). Second, no ADH activity related with ethanol oxidation was found in the placenta (40,46,48) and, third, the different water content in mothers and fetus (20,38) could favor the passage of ethanol from the mother to the fetus. Other authors suggest that the ethanol concentration in fetal fluids, especially in amniotic fluid, could be related with the contribution of the fetal urine to the formation of amniotic fluid at the end of pregnancy, because the ethanol excreted through fetal urine comes from fetal blood and returns to the amniotic fluid (22). Moreover, at the end of pregnancy, the fetus develops the sucking reflex, which could explain the high ethanol levels found in the intragastric fluid compared with maternal blood (up to 200%). This result could be related with the structural and functional alterations found in the perinatal intestine exposed to ethanol in utero (7,32,37). Consequently, this should be taken into account in determining the sensitivity of different fetal tissues to the ethanol concentration involved.

Significantly lower levels of liver ADH activity were found in pregnant animals in the alcohol group. Surprisingly, in virgin animals from the alcohol group, at the same age and period of chronic ethanol treatment as pregnant animals, no decrease in ADH activity was observed, although other authors working at the same pH found lower ADH activities in nonpregnant animals after chronic ethanol treatment (19, 29,56). These differences in the ADH activity were not related with the liver ADH isoenzyme distribution, because this did not show any change with pregnancy or the chronic ethanol treatment. Other liver parameters, such as protein and zinc content, do not help to explain the general decrease in ADH activity in alcohol-treated animals. Some authors have described a deficiency in serum and liver zinc levels after chronic ethanol intake in rats (3,13) and humans (45), but in our model, in agreement with other authors (17,21), no deficiency was found in the alcohol group or in the fibre diet group of adults or offspring. Moreover, in agreement with other authors (6,23,31,47,50,54), and with our own previous studies (52), the increase observed in ethanol clearance after chronic ethanol intake (metabolic tolerance), does not appear to be related to changes in liver ADH activity (29,52). Therefore, although this liver enzyme is the most important in ethanol metabolism (28), it is not a limiting factor to ethanol elimination and it does not fully explain the metabolic tolerance to ethanol (29,52), although the role of ADH as regulator of ethanol elimination is controversial. Furthermore, the malnutrition associated with chronic ethanol intake, represented in our study by the fibre diet group, did not develop changes in ADH activity, as other authors describe using different chronic models (4,8).

The fact that ADH activity at 0 days of pregnancy is higher than that found in 1-month older virgin rats is related to the known decrease in liver ADH activity with the age of the animal (11,13).

In the offspring, no difference was found in liver ADH

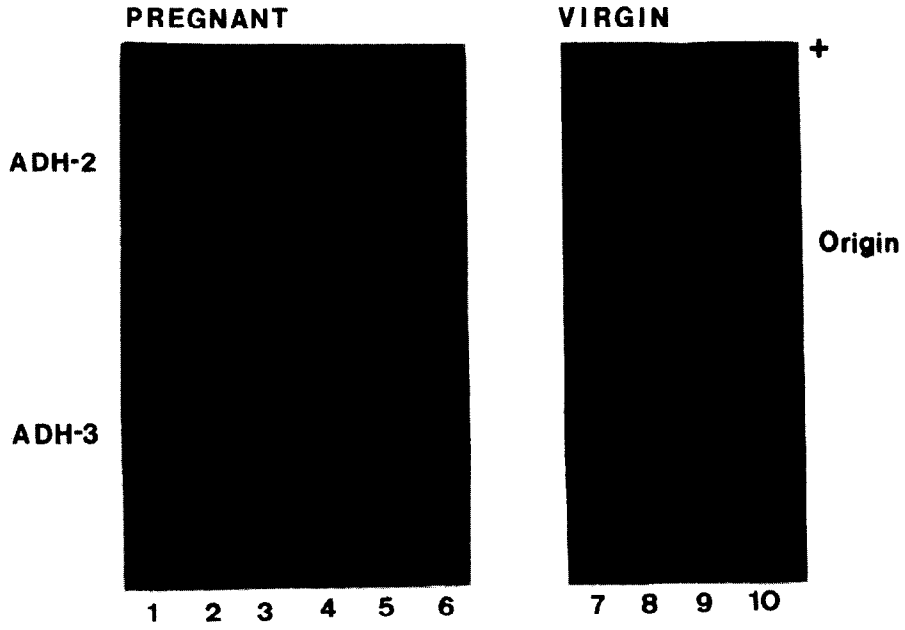

FIG. 3. Starch gel electrophoresis of rat liver ADH isoenzymes: 21-day-old fetus from alcohol (lane 1) and control (lane 4) pregnant rats. Pregnant rats from alcohol (lanes 2 and 3) and control (lanes 5 and 6) groups. Virgin rats from alcohol (lanes 7 and 8) and control (lanes 9 and 10) groups.

activity between groups. In general, liver ADH activity was low in the 21-day-old fetus and in pups at birth, ranging between 10% and 25% of that of adults (virgin animals), respectively. The low ADH activity in fetal liver (42,46,48,56) and the absence of ADH activity metabolizing ethanol in placenta (40,46,48) indicate that maternal ethanol metabolization regulates the elimination of ethanol from both maternal and fetal compartments during late pregnancy (52).

We can deduce that the association of pregnancy with chronic alcoholism significantly modifies the levels of ADH activity, but this is not related to changes in the distribution of the hepatic isoenzymes, or to the zinc content of the tissue.

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