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ALCOHOL DEHYDROGENASE ISOENZYMES IN RAT DEVELOPMENT

EFFECT OF MATERNAL ETHANOL CONSUMPTION

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Abstract—The alcohol dehydrogenase (ADH) isoenzymes (alcohol:NAD oxidoreductase, EC 1.1.1.1) of classes I, III and IV were investigated by activity and starch gel electrophoresis analyses during rat ontogeny. Class I was studied in the liver, class III in the brain and class IV in the stomach and eyes. Classes I and IV exhibited very low activity during the fetal period, reaching 12% and 3%, respectively, of the adult value at birth. Class III was relatively more active in the fetus, with 38% of the adult activity at birth. In the three cases, activity increased after birth and adult values were found around day 20 (classes I and III), day 39 (stomach class IV) and after day 91 (eye class IV). The very low activity of the isoenzymes responsible for ethanol oxidation, i.e. liver class I and stomach class IV, in the fetus demonstrates that metabolism of ethanol during gestation is essentially performed by the maternal tissues. Development of ADH isoenzymes were also studied in the offspring of rats exposed to an alcoholic liquid diet. Activities of liver class I and stomach class IV were severely reduced: they were only 30% and 50%, respectively, of the control values. In contrast, eye class IV activity did not change and brain class III showed a 30% increase. Moreover, the concentration of liver soluble protein exhibited a 1.3–1.5-fold increase with respect to control animals. The effects on activities and liver protein were more pronounced in the adult than in the perinatal period, and they seem irreversible since normal values were not recovered after 6 weeks of feeding with a non-alcoholic diet. The low activities of the alcohol-oxidizing isoenzymes indicate that maternal ethanol consumption results in an impaired ethanol metabolism of the offspring.

Alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1, ADH‡) of mammals consists of several isoenzymes, active towards a variety of alcohols and aldehydes, which represent the most important metabolic system for ethanol elimination. ADH isoenzymes have been grouped in four classes, based on their kinetic and structural properties [1–4]. Class I is the group of isoenzymes, essentially hepatic, with low K_m for ethanol. Class II has been described in liver and exhibits a high K_m for ethanol. Class III is detected in all organs, is specific for long chain alcohols and exhibits glutathione-dependent formaldehyde dehydrogenase activity [5]. Class IV ADH has been described in the digestive tract and external epithelia of rat and shows specificity for medium and long chain alcohols. It has been suggested that the new isoenzyme, σ-ADH, recently characterized in the human stomach, is homologous to rat class IV [6]. The four isoenzyme classes differ from each other in 30–40% of the amino acid sequence, while isoenzymes within a single class (i.e. human class I) exhibited less than 10% difference [3, 4].

Isoenzymes of the classes I, III and IV are well known for the rat species [2, 4]. Class I is responsible for hepatic ethanol metabolism and class IV has a role in gastric ethanol oxidation, whereas class III is involved in fatty alcohol and formaldehyde metabolism but its function in ethanol oxidation is probably minimal [2, 7]. Determination of the variation in ADH activity during development is essential to estimate changes in alcohol metabolism and toxicity at different ontogenic stages. Previous reports on this subject in the rat species have focused on liver class I development [8–13]. A reinvestigation is, however, necessary with regard to the present knowledge of the three different isoenzyme classes, differently distributed in the rat organs and with distinct physiological functions. In the present report we have studied the changes in activity and isoenzymatic pattern of the three ADH classes throughout the development of the rat liver, stomach, eyes, brain and placenta. We also present data on the ADH activity changes during the development of the offspring of mothers fed an alcoholic diet. We used a model in which the female rat was fed a liquid diet with 5% ethanol during 1 month before pregnancy, and throughout gestation and lactation. This animal model has been proven to reproduce in the offspring some symptoms and alterations characteristic of children with fetal alcohol syndrome [14, 15].

MATERIALS AND METHODS

Materials. NAD (grade III and grade AA-1), dithiothreitol, starch, phenazine methosulphate, nitrobluetetrazolium and pyruvic acid were pur-

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‡ Abbreviation: ADH, alcohol dehydrogenase.

chased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Substrates and buffers were obtained from Merck (Darmstadt, F.R.G).

Rat tissues. Adult virgin female Wistar rats with an average initial body weight of 150–200 g were used. All animals were acclimated to laboratory conditions for at least 1 week before treatment. They were housed in groups of four to five animals per cage and maintained under controlled conditions of light and dark (12/12 hr), temperature (23 ± 1°), and humidity (60%). They were divided randomly into three groups according to diet: the control group received standard solid rat chow and tap water *ad. lib.*; the alcoholic animals received an ethanol liquid diet (5% w/v) in which ethanol provided 36% of the total calories [16]; the pair-fed group was given a similar liquid diet for the same period, except that maltose-dextrin replaced ethanol isocalorically. All animals received fresh food daily. The ethanol content of the alcohol liquid diet was increased gradually from 1 to 5% during a period of 1 week. The alcoholic and pair-fed females were maintained on their diets for 4–5 weeks before mating. No differences were observed in body weight between both groups at the end of treatment.

Mating was determined by the presence of sperm in vaginal lavage, defined as occurring day zero of gestation. Pregnant rats were housed in individual cages and kept on their respective diets. Offspring were separated from mothers on day 25 postnatally and their diet was changed to standard rat chow. In the alcoholic group, mothers were maintained on alcoholic diet throughout the experiment such that offspring were exposed to ethanol during their fetal and suckling periods. On different days of gestation, pregnant rats and fetuses were killed by decapitation, and placenta, fetal liver, stomach, brain and eyes were dissected out immediately. On different days of postnatal development, pups from the three diet groups were killed and the organs were dissected out. Both male and female offspring were used. All animals were sacrificed at the same time of day (around 9:00 a.m.) to avoid circadian variation. Stomachs were cut, cleaned and washed in ice-cold distilled water. All organs were frozen in liquid nitrogen and stored at −80°. ADH activities of the stored material did not differ significantly from those of fresh tissues.

Sample preparation. Prior to analysis, organs were thawed, cut in small pieces and homogenized in 50 mM sodium pyrophosphate pH 8.8, 0.5 mM dithiothreitol, using a 1:2 w/v or 1:3 w/v dilution, with an Ystral (Dottingen, F.R.G.) homogenizer. The homogenates were centrifuged at 27,000 *g* for 1 hr at 4°. Supernatants were used for ADH activity determinations and electrophoretic analysis. Protein concentration was determined by the Coomassie blue method [17] using bovine serum albumin as a standard.

Electrophoresis. Starch gel electrophoresis of tissue homogenates (10 μL) was carried out at 4°, pH 7.6, on horizontal gels, for 5 hr at 720 V using a Multiphor instrument (Pharmacia LKB, Uppsala, Sweden) [2]. The gels were stained for activity using 0.1 M 2-buten-1-ol as a substrate.

Enzyme assays. ADH activity of tissue homogenates was determined spectrophotometrically at 25° as the increase in absorbance at 340 nm in a Cary 219 instrument. Activity assays were performed in 0.1 M glycine/NaOH pH 10.0, 4 mM NAD. The substrates were 33 mM ethanol for the class I ADH, 1 M ethanol for the class IV ADH, and 1.5 mM octanol for the class III ADH. Activities are expressed as units. One unit corresponds to one μmole of NADH formed per minute. Results are presented as the mean ± SEM of 3–5 experiments performed with different animals. The ANOVA and Student's *t*-tests were used for statistical analysis.

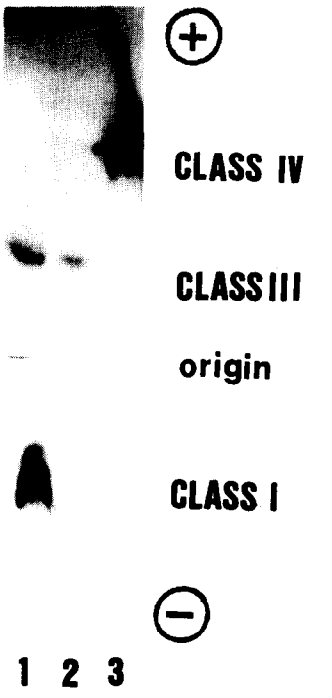

Fig. 1. Separation of ADH isoenzymes by starch gel electrophoresis of homogenates from rat liver (lane 1), brain (lane 2) and stomach (lane 3), stained for 2-buten-1-ol activity.

RESULTS

Development of ADH isoenzymes

Analysis of the isoenzymes during the development of the rat was performed by starch gel electrophoresis and by determination of the ADH activity specific for each isoenzymatic class. Starch gel electrophoresis served to identify each isoenzyme present in the sample, while activity was used to measure the amount of active enzyme.

Because of the differences in localization and in enzymatic activity of the ADH isoenzymes in the rat tissues, several organs had to be used (Fig. 1). Liver and stomach were chosen because of their high content of class I and class IV, respectively, and because of their intrinsic importance in ethanol metabolism. The eyes were also used for class IV

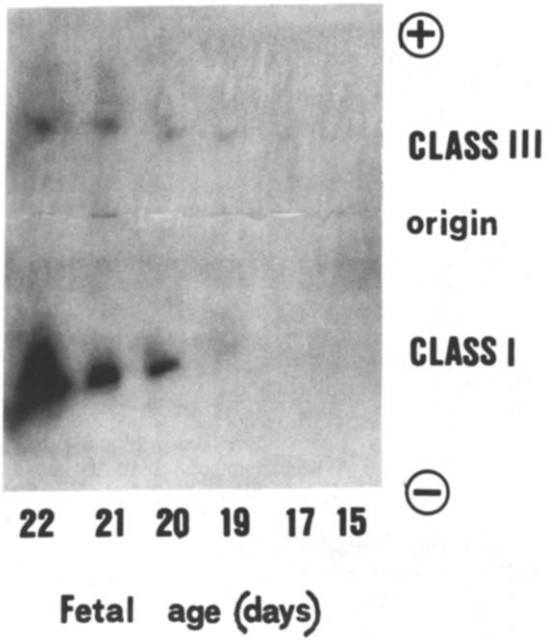

Fig. 2. Starch gel electrophoresis of homogenates from fetal rat liver of different days of gestation, stained for 2-buten-1-ol activity.

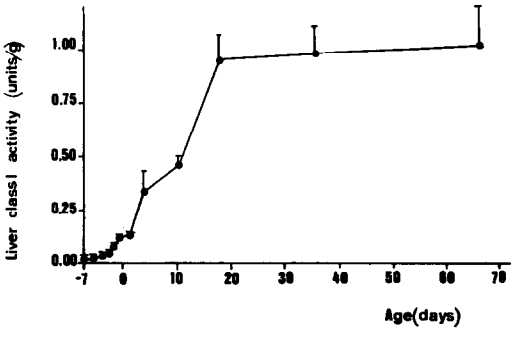

Fig. 3. Changes in liver class I ADH activity during rat development. Each point represents the mean of three to five values from different animals ± SEM.

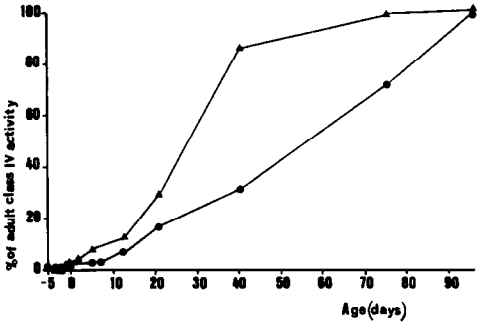

Fig. 4. Relative changes in class IV ADH activity during the development of rat stomach (▲) and eyes (●). Each point represents the mean of three to five values from different animals. One hundred per cent activity corresponds to 1.2 U/g and 0.9 U/g of stomach and eyes, respectively.

analysis because of their high activity and to serve as a comparison for the stomach. Although all these organs also contained class III, the activity of this isoenzyme was not significant under the conditions used for the liver class I assay, while the amount of class III in the stomach and eye was very small.

The study of class III development was essentially performed in the brain because of the importance of this organ in the pathology of alcohol and the possible role of class III in brain fatty alcohol metabolism [18]. The placenta was also analysed for class III activity, whereas detection of the enzyme by starch gel electrophoresis was performed in all organs studied.

Starch gel electrophoresis of homogenates from fetal livers of different ages showed the progressive appearance of classes I and III isoenzymes (Fig. 2). With this technique class I was detected first on day 19, although activity could be detected as early as day 15, being 2% of the adult activity. At birth (day 22) class I exhibited 12% of the adult activity (Fig. 3). After day 20 of gestation class I activity increased rapidly up to day 20 postnatally. The activity values remained steady after that day.

Stomach class IV activity was detected first on day 21 of fetal development (Fig. 4) and increased progressively up to day 39, when adult activity had already been reached. In the eyes, a very small class IV activity was detected around birth. An activity

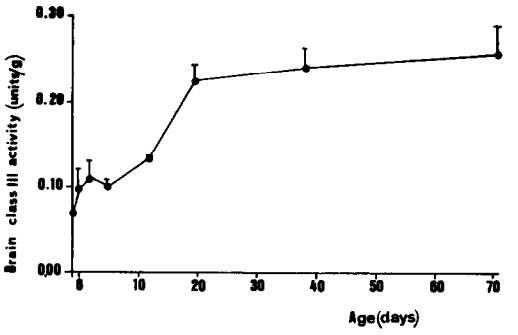

Fig. 5. Changes in class III ADH activity of the whole brain during rat development. Each point represents the mean of three to five values from different animals ± SEM.

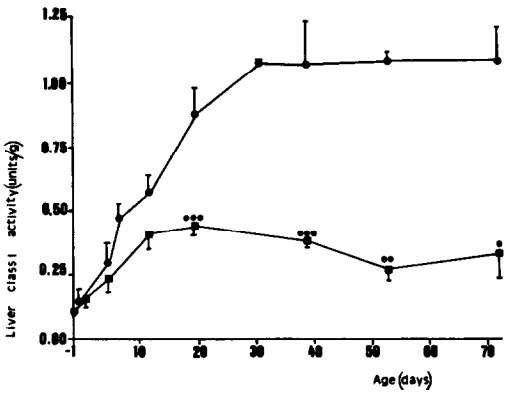

Fig. 6. Changes in liver class I ADH activity during the development of pair-fed (●) and alcoholic (■) rats. Each point represents the mean of three to five values from different animals ± SEM. *P < 0.03, **P < 0.015, ***P < 0.005.

burst took place after day 7 postnatally and the activity continued increasing until the last day studied (day 91). Both organs, the stomach and the eyes, showed at birth only 3% of the adult activity.

Class III was present in all organs from the first days of development analysed (Fig. 2). Although the amount of class III in brain was small, its activity in the fetus was significant as compared with the adult activity, and 38% of the adult activity was already present at birth (Fig. 5). Activity increased subsequently, reaching adult values on day 20. The placenta showed a small amount of class III (0.01 U/g) that remained practically constant during the last five days of pregnancy. Similarly to the human tissue [19], no other ADH isoenzyme was detected in the rat placenta.

Effect of maternal exposure to ethanol on the development of ADH isoenzymes in the offspring

Offspring of alcoholic rats were exposed to ethanol during gestation and lactation. After weaning (day 25), pups received standard solid chow. Starch gel electrophoresis of organs from fetuses and pups did not reveal any difference in mobility and number of isoenzymes, as compared to control rats.

ADH activity analysis of the pair-fed group did not show any significant difference from the activities of the control group. Therefore, in presenting the results concerning the alcoholic animals, only the activities of the pair-fed group will be indicated for comparison. In Fig. 6 the activities of liver class I of the alcoholic and pair-fed groups are compared. Exposure to ethanol resulted in a strong negative effect on the development of the enzyme activity expressed in U/g of tissue. Activity of the alcoholic offspring reached only 30% of the pair-fed controls. Stomach class IV activity was also reduced by 50% when the offspring were exposed to ethanol (Fig. 7). In contrast, ocular class IV was not affected significantly by ethanol (result not shown). Brain class III had an opposite behaviour to both liver class I and stomach class IV, since ethanol apparently induced a 30% increase in the isoenzyme activity in cerebral tissue (Fig. 8).

The effect of exposure to ethanol on the concentration of soluble proteins was determined in

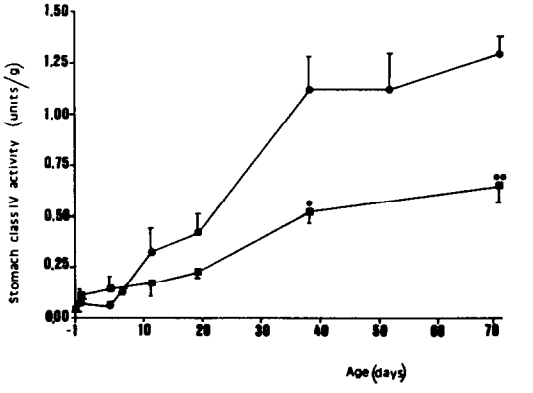

Fig. 7. Changes in stomach class IV ADH activity during the development of pair-fed (●) and alcoholic (■) rats. Each point represents the mean of three to five values from different animals ± SEM. *P < 0.03, **P < 0.01.

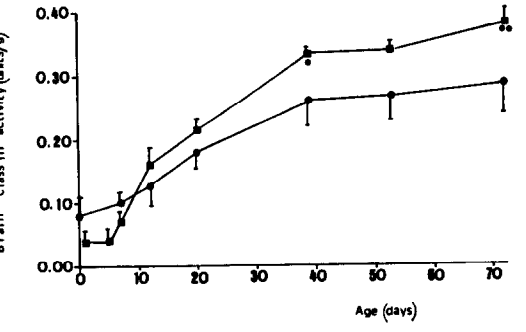

Fig. 8. Changes in brain class III ADH activity during the development of pair-fed (●) and alcoholic (■) rats. Each point represents the mean of three to five values from different animals ± SEM. *P < 0.03, **P < 0.02.

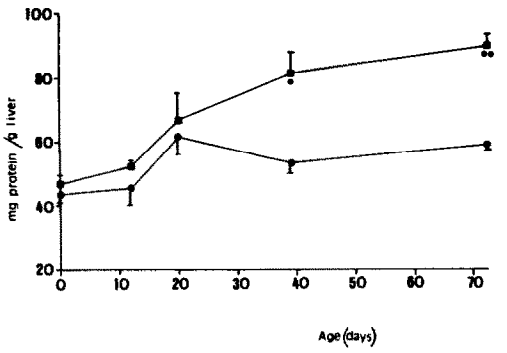

Fig. 9. Changes in protein concentration of liver homogenates during the development of pair-fed (●) and alcoholic (■) rats. Each point represents the mean of three to five values from different animals ± SEM. *$P < 0.05$, **$P < 0.005$.

the liver, stomach, eyes and brain, but only the liver showed a significant effect (Fig. 9). Alcoholic offspring exhibited a pronounced increase (by a factor of 1.3–1.5) of hepatic protein concentration as compared to pair-fed controls. The differences were more significant during the adult than the perinatal period.

DISCUSSION

The development pattern of rat class I ADH presented here agrees well with previous reports [8–13]. Fetal activity is very small; it starts to increase by day 21 of gestation, is 12% of the adult activity at birth and reaches the adult value by day 20 postnatally. It has been demonstrated previously by immunoenzymatic analysis that the increase in activity during development corresponds to an increase in the amount of enzyme protein [11]. The development activity pattern of rat class I ADH is similar to those reported previously for the class I isoenzymes of other mammals: man [20, 21], ewe [22], mouse [23–25] and guinea pig [26].

ADH of class IV has an extremely low activity during rat fetal life and the first postnatal days in both organs studied, the stomach and the eyes. The activity burst in the stomach starts immediately after birth and continues up to day 39, when adult activity seems to have been reached. Class IV activity of the eyes begins to increase after day 7 postnatally. The increase is practically linear until the last day of the experiment (day 90). The time difference in the beginning of the activity burst may be related to a time difference in the start of functionality of the two organs. The stomach is already functional immediately after birth whereas opening of the eyes occurs several days later. A similar result has been reported for the aldehyde dehydrogenase isoenzyme characteristic of the mouse cornea, which is detected on day 8 in ocular tissues but is already present at birth in the lung and skin [27]. The ocular isoenzyme of mouse ADH, which is probably homologous to the rat class IV, also shows an increase in activity at the end of the first week [25].

It has been estimated that liver class I accounts for 90% of ethanol dehydrogenase activity in the rat, using 33 mM ethanol at pH 7.5, whereas stomach class IV, which is active at higher ethanol concentrations ($K_m = 5$ M), may make a significant contribution in the oxidation of ingested ethanol [7]. Here, we have demonstrated that liver class I and stomach class IV activities are very low before birth, indicating that ethanol metabolism during gestation is performed essentially by the maternal tissues. After birth, the increase in both activities would allow the elimination of the ethanol ingested by the pup from the mother's milk during the suckling period. Thus, blood ethanol levels in pups (0.3–1.0 mM) are much lower than in maternal milk (17–20 mM) [28] indicating an active ethanol metabolism in the offspring. The metabolic rate is, however, lower than in adults [13].

Enzymes have been grouped in different clusters depending on their emergence during ontogeny [29]. ADHs of classes I and IV belong to the cluster that shows little fetal activity with an abrupt increase in activity during the perinatal period (Figs 3 and 4). However, ADH class III shows a different development pattern (Fig. 5): it is detected in the early stages of development in all the organs studied (brain, liver, placenta, stomach, eye) suggesting an important role from the first days of life, related to formaldehyde elimination and/or fatty alcohol metabolism. In the brain, 38% of the adult activity is already present at birth. Activity reaches the adult value on day 20, when the brain is already mature.

A striking effect of maternal exposure to ethanol on the ADH isoenzymes is the very low liver class I activity reached in the offspring as compared with the control animals (Fig. 6). Differences are not significant before day 10 postnatally but reduction of the activity is very severe afterwards, reaching 70%. The normal activity is not recovered after weaning, when pups are no longer exposed to ethanol. The effect appears, therefore, to be irreversible, at least during the 70 days studied. A previous work, using the same animal model, reported also a decrease in class I activity of the alcohol-exposed offspring [10]. In contrast, another report did not detect any activity change [12]. The only important difference between the experimental model used in the latter study and ours is that in the latter study the mother was not exposed to ethanol before pregnancy. In this regard, a recent work shows a significant decrease in class I activity in the offspring of mothers exposed to ethanol for a period of only 1 month before mating [30]. Taken together these results suggest that important effects of ethanol on liver ADH class I activity take place during the very early stages of gestation.

The severe and irreversible effect of maternal ethanol exposure on class I activity of the offspring suggests that the primary effect is on an important regulatory mechanism of the enzyme. Much recent evidence indicates that rat ADH class I is under hormonal control, performed to a significant degree by the hypothalamus–hypophysis axis [31, 32]. The impaired function of this hormonal system, which is

known to be altered by prenatal ethanol [14, 33], may be the explanation for the low class I activity found in the developing and adult offspring of ethanol-exposed rats.

Maternal ethanol exposure causes an important decrease in the stomach class IV activity, but no changes were detected in the ocular class IV. A possible explanation is a distinct regulatory mechanism for class IV expression (or turnover) in each organ, each differently affected by ethanol. Alternatively, the stomach enzyme of the pup could be more altered because it is in contact with a higher ethanol concentration than that of the eyes during suckling. A decrease in stomach ADH activity is also detected in adult rats with chronic oral intake of ethanol [34].

As discussed previously, most of the ethanol oxidation in the rat is performed by liver class I, with a contribution in the gastric ethanol metabolism by class IV [7, 34]. The severe and irreversible decrease in both enzymes by maternal ethanol consumption will result in impaired ethanol metabolism of the offspring, since ADH is the most important limiting factor in ethanol elimination [35]. However, that there is a contribution from the microsomal oxidizing system, which may increase in the ethanol-treated animals [36], cannot be excluded.

In contrast to the decrease in classes I and IV, maternal ethanol intake causes an increase in brain class III, suggesting a different mechanism for the ethanol action on this isoenzyme in the brain. Such an activity increase is not unique to brain ADH class III, since the activity of other enzymes, like brain γ-glutamyl transpeptidase [37], superoxide dismutase and catalase [38], also increases in the offspring of ethanol-treated mothers.

The increase in liver protein concentration induced by chronic alcohol intake has been reported consistently in adult rats [39], with a 1.2-fold increase with respect to controls. In our experiments this increase is even higher (1.3–1.5 times) suggesting that exposure to ethanol during fetal and postnatal life has a more severe effect on liver protein than during the adult period. Moreover, in our experimental animal model protein accumulation appears to be irreversible, since the phenomenon is even more pronounced in adult rats, after 6 weeks of non-alcoholic standard chow diet. We have demonstrated previously that prenatal alcohol exposure induces a significant increase in the ratios of liver/body weight and hepatic protein level/body weight [40], and causes an increase in hepatocyte and mitochondrial volume [41]. Another important effect of ethanol treatment on the newborn rat hepatocytes is a striking disorganization of the Golgi apparatus [41, 42] and several dysfunctions in the glycoprotein metabolism of this organelle [40, 43]. Therefore, alterations in protein trafficking in the Golgi apparatus, together with a possible functional impairment of microtubule-dependent events [39], would result in inhibition of protein secretion and in the accumulation of the proteins observed in the liver of rats exposed to alcohol.

In summary, our results demonstrate that the ADH isoenzymes responsible for ethanol elimination (classes I and IV) exhibit extremely low activities in the rat fetus which will depend, therefore, on the maternal metabolic systems to eliminate ethanol. Development of liver class I and stomach class IV is greatly impaired by maternal consumption of ethanol. The decrease in activity appears irreversible and it will result in a low capacity for ethanol metabolism in the offspring of alcoholic rats. A low ADH activity could be an explanation for the high sensitivity to ethanol reported in some patients with fetal alcohol syndrome [44].

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REFERENCES

1. Vallee BL and Bazzone TJ, Isoenzymes of human liver alcohol dehydrogenase. *Isozymes Curr Top Biol Med Res* **8**: 219–244, 1983.
2. Julià P, Farrés J and Parés X, Characterization of three isoenzymes of rat alcohol dehydrogenase. Tissue distribution and physical and enzymatic properties. *Eur J Biochem* **162**: 179–189, 1987.
3. Jörnvall H, von Bahr-Lindström H and Höög J-O, Alcohol dehydrogenases—structure. In: *Human Metabolism of Alcohol, Vol. II* (Eds. Crow KE and Batt RD), pp. 43–64. CRC Press, Boca Raton, FL, 1989.
4. Parés X, Moreno A, Cederlund E, Höög J-O and Jörnvall H, Class IV mammalian alcohol dehydrogenase. Structural data of the rat stomach enzyme reveal a new class well separated from those already characterized. *FEBS Lett* **277**: 115–118, 1990.
5. Koivusalo M, Baumann M and Uotila L, Evidence for the identity of glutathione-dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Lett* **257**: 105–109, 1989.
6. Moreno A and Parés X, Purification and characterization of a new alcohol dehydrogenase from human stomach. *J Biol Chem* **266**: 1128–1133, 1991.
7. Boleda MD, Julià P, Moreno A and Parés X, Role of extrahepatic alcohol dehydrogenase in rat ethanol metabolism. *Arch Biochem Biophys* **274**: 74–81, 1989.
8. Räihä NCR, Koskinen M and Pikkarainen P, Developmental changes in alcohol-dehydrogenase activity in rat and guinea-pig liver. *Biochem J* **103**: 623–626, 1967.
9. Sjöblom M, Pilström L and Morland J, Activity of alcohol dehydrogenase and acetaldehyde dehydrogenases in the liver and placenta during the development of the rat. *Enzyme* **23**: 108–115, 1978.
10. Guerri C and Grisolía S, Effects of prenatal and postnatal exposure of rats to alcohol: changes in (Na^+-K^+) ATPase. *Pharmacol Biochem Behav* **17**: 927–932, 1982.
11. Lad PJ, Shoemaker WJ and Leffert HL, Developmental changes in rat liver alcohol dehydrogenase. *Dev Biol* **105**: 526–529, 1984.
12. Rovinski B, Hosein EA, Lee H, Hin GLY and Rastogi NK, Hepatotoxicity of maternal ethanol consumption in rat offspring: an assessment with a study of the ontogenetic development of ethanol-oxidizing systems. *Alcohol Drug Res* **7**: 195–205, 1987.
13. Zorzano A and Herrera E, Decreased *in vivo* rate of ethanol metabolism in the suckling rat. *Alcohol Clin Exp Res* **13**: 527–532, 1989.
14. Guerri C, Esquifino R, Sanchis R and Grisolía S,

Growth, enzymes and hormonal changes in offspring of alcohol-fed rats. In: *Mechanisms of alcohol damage in utero, Ciba Found Symp* **105**: 85–102, 1984.
15. Sanchis R, Sancho-Tello M and Guerri C, The effects of chronic alcohol consumption on pregnant rats and their offspring. *Alcohol Alcohol* **21**: 295–305, 1986.
16. Lieber CS and DeCarli L, Animal models of ethanol dependence and liver injury in rats and baboons. *Fed Proc* **35**: 1235–1236, 1976.
17. Bradford M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
18. Giri PR, Linnoila M, O'Neill JB and Goldman D, Distribution and possible metabolic role of class III alcohol dehydrogenase in the human brain. *Brain Res* **481**: 131–141, 1989.
19. Parés X, Farrés J and Vallee BL, Organ specific alcohol metabolism: Placental χ-ADH. *Biochem Biophys Res Commun* **119**: 1047–1055, 1984.
20. Pikkarainen PH and Räihä NCR, Development of alcohol dehydrogenase activity in the human liver. *Pediat Res* **1**: 165–168, 1967.
21. Smith M, Hopkinson DA and Harris H, Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann Hum Genet* **34**: 251–271, 1971.
22. Clarke DW, Smith GN, Patrick J, Richardson B and Brien JF, Activity of alcohol dehydrogenase and aldehyde dehydrogenase in maternal liver, fetal liver and placenta of the near-term pregnant ewe. *Dev Pharmacol Ther* **12**: 35–41, 1989.
23. Balak KJ, Keith RH and Felder MR, Genetic and developmental regulation of mouse liver alcohol dehydrogenase. *J Biol Chem* **257**: 15000–15007, 1982.
24. Smolen TN, Smolen A and van de Kamp JL, Developmental profile of hepatic alcohol and aldehyde dehydrogenase activities in long-sleep and short-sleep mice. *Alcohol* **7**: 69–74, 1990.
25. Rout UK and Holmes RS, Postnatal development of mouse alcohol dehydrogenases: agarose isoelectric focusing analyses of the liver, kidney, stomach and ocular isozymes. *Biol Neonate* **59**: 93–97, 1991.
26. Card SE, Tomkins SF and Brien JF, Ontogeny of the activity of alcohol dehydrogenase and aldehyde dehydrogenases in the liver and placenta of the guinea pig. *Biochem Pharmacol* **38**: 2535–2541, 1989.
27. Rout UK and Holmes RS. Postnatal development of mouse aldehyde dehydrogenases. Agarose-isoelectric focusing analyses of the heart, lung, skin and ocular isozymes. In: *Biomedical and Social Aspects of Alcohol and Alcoholism* (Eds. Kuriyama K, Takada A and Ishii H), pp. 139–143. Elsevier Science Publishers, Amsterdam, 1988.
28. Guerri C and Sanchis R, Alcohol and acetaldehyde in rat's milk following ethanol administration. *Life Sci* **38**: 1543–1556, 1986.
29. Greengard O, Enzymatic differentiation in mammalian tissues. *Essays Biochem* **7**: 159–205, 1971.
30. Ledig M, Misslin R, Kopp P, Vogel E, Tholey G and Mandel P, Alcohol exposure before pregnancy: biochemical and behavioral effect on the offspring of rats. *Pharmacol Biochem Behav* **36**: 279–285, 1990.
31. Crabb DW, Hormonal regulation of alcohol dehydrogenase activity. In: *Human Metabolism of Alcohol, Vol. II* (Eds. Crow KE and Batt RD), pp. 31–40. CRC Press, Boca Raton, FL, 1989.
32. Mezey E, Potter JJ, Mishra L, Sharma S and Janicot M, Effect of insulin-like growth factor I on rat alcohol dehydrogenase in primary hepatocyte culture. *Arch Biochem Biophys* **280**: 390–396, 1990.
33. Esquifino AI, Sanchís R and Guerri C, Effect of prenatal alcohol exposure on sexual maturation of female rat offspring. *Neuroendocrinology* **44**: 483–487, 1986.
34. Julkunen RJK, Di Padova C and Lieber CS, First pass metabolism of ethanol—a gastrointestinal barrier against the systemic toxicity of ethanol. *Life Sci* **37**: 567–573, 1985.
35. Lumeng L, Bosron WF and Li TK, Quantitative correlation of ethanol elimination rates *in vivo* with liver alcohol dehydrogenase activities in fed, fasted and food-restricted rats. *Biochem Pharmacol* **28**: 1547–1551, 1979.
36. Lieber CS, Hepatic, metabolic and toxic effects of ethanol: 1991 update. *Alcohol Clin Exp Res* **15**: 573–592, 1991.
37. Reyes E, Rivera JM, Saland LC and Murray HM, Effects of maternal administration of alcohol on fetal brain development. *Neurobehav Toxicol Teratol* **5**: 263–267, 1983.
38. Iborra FJ, Azorín I, Fornas E, Sáez R, Renau-Piqueras J and Guerri C, Effect of chronic and fetal ethanol exposure on lipid peroxidation in rat brain. *Alcohol Alcohol* **26**: 246, 1991.
39. Baraona E and Lieber CS, Effects of alcohol on hepatic transport of proteins. *Annu Rev Med* **33**: 281–292, 1982.
40. Guerri C, Guasch R and Renau-Piqueras J, Chronic and prenatal alcohol treatments induce alteration in the glycosilation of protein in the trans-Golgi apparatus. *Alcohol Clin Exp Res* **14**: 356, 1990.
41. Renau-Piqueras J, Gómez-Perreta C, Guerri C and Sanchis R, Qualitative and quantitative ultrastructural alterations in hepatocytes of rats prenatally exposed to ethanol with special reference to mitochondria, Golgi apparatus and peroxisomes. *Virchows Arch* **405**: 237–251, 1985.
42. Renau-Piqueras J, Miragall F, Guerri C and Baguena-Cervellera R, Prenatal exposure to alcohol alters the Golgi apparatus of newborn rat hepatocytes: a cytochemical study. *J Histochem Cytochem* **35**: 221–228, 1987.
43. Renau-Piqueras J, Sancho-Tello M, Báguena-Cervellera R and Guerri C, Prenatal exposure to ethanol alters the synthesis and glycosylation of proteins in fetal hepatocytes. *Alcohol Clin Exp Res* **113**: 817–823, 1989.
44. Streissguth AP, Long-term consequences of fetal alcohol exposure in humans. In: *International Workshop on Alcohol and Fetal Development*, Valencia, Spain, October 4–5, 1990.