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Ethanol Consumption by the Nursing Mother Induces Cytochrome P-4502E1 in Neonatal Rat Liver[1]

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ABSTRACT

Cytochrome P-4502E1 (P-4502E1) is not present in fetal rat liver because activation of the gene occurs shortly after birth. Ethanol is an inducer of P-4502E1 in adult rats. Studies were carried out to evaluate whether transplacental induction of P-4502E1 by ethanol can occur after oral consumption of ethanol by the pregnant mother. Because ethanol can be excreted in breast milk, the possible induction of P-4502E1 in neonatal liver when ethanol was consumed during the gestational and neonatal period by the mother was also determined. Pregnant rats received control or an ethanol-containing liquid diet starting on the 9th day of gestation and were killed on the 17th day or 21st day of gestation or allowed to deliver. The rats continued on their respective diets for the first 2 weeks of the neonatal period. P-4502E1 messenger RNA (mRNA), protein or catalytic activity was not detectable in fetal liver and was not induced in the fetuses from the ethanol-consuming mothers. Transplacental induction of P-4502E1 by ethanol did not occur in this model. Induction by ethanol of P-4502E1 protein and catalytic activity but not mRNA occurred in maternal liver. P-4502E1 mRNA, protein and catalytic activity were detected shortly after birth and increased over the 2-week neonatal period. The P-4502E1 content and oxidation of *p*-nitrophenol or dimethylnitrosamine by hepatic microsomes from neonates of mothers consuming the ethanol diet were increased 2- to 3-fold compared with controls; however, P-4502E1 mRNA levels were not elevated. These results indicate that consumption of ethanol during the gestational and neonatal period can result in induction of P-4502E1 in hepatic microsomes of neonates, suggesting that the ethanol present in the mother's milk is transferred to the newborn and is capable of inducing the P-4502E1 by a post-transcriptional mechanism. Such a mechanism of induction, which can occur immediately after birth, may be of toxicological significance to the newborn in view of the catalytic properties associated with P-4502E1.

The content of cytochrome P-450 in the fetal livers of rats and rabbits is low because transcriptional activation of cytochrome P-450 genes occur shortly after birth (Gillette and Stripp, 1975; Neims *et al.*, 1976; Mannering, 1985; Bonfils *et al.*, 1990; Giachelli and Omiecinski, 1986, 1987; Cresteil *et al.*, 1986). P-4502E1 mRNA or protein was not detectable in rat fetal liver (Song *et al.*, 1986; Umeno *et al.*, 1988; Hong *et al.*, 1987a; Ueno and Gonzalez, 1990; Peng *et al.*, 1991) because activation of the P-4502E1[1] gene in the liver occurs within hours after birth (Umeno *et al.*, 1988). Once activated, the P-4502E1 mRNA levels remain relatively constant or slowly increase (Umeno *et al.*, 1988; Hong *et al.*, 1987a). Activation of the P-4502E1 gene is accompanied by demethylation of cytosine residues located at the 5′ end of the gene (Umeno *et al.*, 1988). Increases in P-4502E1 mRNA levels are associated with corresponding elevations in the content of P-4502E1 protein and oxidation of effective substrates for P-4502E1 catalytic activity, such as aniline and DMN (Song *et al.*, 1986; Hong *et al.*, 1987a; Yoo *et al.*, 1987; Thomas *et al.*, 1987).

P-4502E1 activates a variety of compounds that are of toxicological significance (Koop, 1992; Yang *et al.*, 1990; Guengerich *et al.*, 1991). Induction of P-4502E1 may contribute to the mechanisms by which ethanol damages the liver (Lieber, 1988, 1990). In general, the induction of P-4502E1 protein and catalytic activity by small molecular weight inducing agents, such as ethanol, acetone, isoniazid, pyrazole and 4-methylpyrazole, is not associated with transcriptional activation of the P-4502E1 gene (Song *et al.*, 1986; Hong *et al.*, 1987a; Peng *et al.*, 1991; Johansson *et al.*, 1988). The mechanism of P-4502E1 induction by these agents was suggested to be at the level of stabilization of P-4502E1 protein against degradation (Song *et al.*, 1989; Eliasson *et al.*, 1988). This suggests that inducers, such as ethanol (which work primarily at the level of the protein), would not effectively induce P-4502E1 in rat fetal liver in which P-4502E1 protein is not detectable. However, Hong *et al.* (1987a) reported that transplacental induction of P-4502E1 by acetone could occur when the acetone was administered to the pregnant mother on days 19 and 20 of gestation.

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ABBREVIATIONS: PNP, *p*-nitrophenol; DMN, *N*,*N*-dimethylnitrosamine; P-4502E1, cytochrome P-4502E1.

The possibility that transplacental induction of P-4502E1 by ethanol can occur would appear to be important to evaluate in view of the toxic effects of alcohol to the fetus and the toxicological properties associated with P-4502E1. In the current report, the ability of ethanol to induce P-4502E1 in the fetal liver after its consumption in a liquid diet by the pregnant mother during gestation was determined. Induction of P-4502E1 in the liver of the pregnant mother consuming the ethanol diet was also determined in view of the report by Casazza *et al.* (1990) that there was a progressive loss of P-4502E1 during pregnancy and to validate that sufficient ethanol was consumed to induce P-4502E1 at least in the maternal liver.

Injecting pyrazole or 4-methylpyrazole into rat pups immediately after birth or during the first 2 weeks of the neonatal period resulted in 2- to 4-fold increases in the content of P-4502E1 in hepatic microsomes (Wu and Cederbaum, 1993). These increases were associated with enhanced oxidation of DMN but not with elevated P-4502E1 mRNA levels. Ding *et al.* (1992) recently reported a 3-fold increase in P-4502E2 content (but not P-4502E1) when imidazole was administered to neonatal rabbits on days 8 to 11 after birth. These results indicate that P-4502E can be induced immediately after birth and during the early neonatal period. In view of the induction of P-4502E1 by pyrazole and 4-methylpyrazole or P-4502E2 by imidazole in neonates, it would appear important to validate induction by ethanol in newborns and neonates. Direct administration of ethanol by oral intake or by injection would not be relevant for human neonatal conditions. Because ethanol is excreted in breast milk (Kesaniemi, 1974; Lawton, 1985), we determined whether consumption of ethanol during the gestational and neonatal periods by the mother could result in induction of P-4502E1 in the newborn pups.

Materials and Methods

Pregnant Sprague-Dawley rats, weighing about 250 to 300 g, were purchased from Zivic Miller (Allison Park, PA) and fed normal Purina lab chow (Ralston Purina PMI Feeds, Inc., St. Louis, MO) until the 9th day of gestation. The rats were divided into two groups. One group was fed a high-protein liquid diet containing 6.7% w/v ethanol (Bio-Serve, Frenchtown, NJ) and the other group was fed the high-protein liquid control diet. Some rats in each group were killed on the 17th or 21st day of gestation and the livers were removed from the mothers and their fetuses. Other rats were allowed to deliver; the pups were killed either on the day of birth (generally within 12 hr after birth) or 4, 8 and 14 days after birth. The mothers were killed 14 days after delivery. During this 14-day neonatal period, the mothers continued to consume the same liquid diet that they had consumed during the gestational period. Male and female pups were pooled and not studied separately. The livers were rapidly removed and divided into two parts for each fetus, pup or mother. The livers from several fetuses and pups were pooled together to provide sufficient material for the various analyses. One part of the whole liver or the pooled livers was homogenized and microsomes were prepared by differential centrifugation, suspended in 125 mM KCl with 10 mM potassium phosphate, pH 7.4, and stored at −70°C. The second part of the liver was used to prepare total RNA, which was isolated by an acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987).

Microsomal protein was assayed by the method of Lowry *et al.* (1951); the content of total cytochrome P-450 was determined from the reduced cytochrome P-450 carbon monoxide binding spectrum (Omura and Sato, 1964). The oxidation of DMN was determined in a reaction system containing 100 mM potassium phosphate buffer, pH 7.4, 4 mM DMN, 1 mM NADPH and 1.2 mg of microsomal protein in a final volume of 0.25 ml. Hydroxylation of PNP was determined in a reaction system containing 100 mM phosphate buffer, pH 7.2, 0.2 mM PNP, 1 mM NADPH and 0.6 mg of microsomal protein in a final volume of 0.5 ml. The reactions were initiated by the addition of NADPH and were carried out for 20 min at 37°C. They were terminated by the addition of trichloroacetic acid and formaldehyde or *p*-nitrocatechol formation was determined by standard colorimetric assays (Nash, 1953; Reinke and Moyer, 1985). All values were corrected for zero-time controls, which contained acid before the addition of NADPH.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed using a 7.5% running gel and a 4% stacking gel. Heat-denatured microsomes (10–50 μg, depending on the expected P-4502E1 content) or 5 pmol of P-4502E1 purified from pyrazole-treated rats (Palakodety *et al.*, 1988) were loaded onto the gel and electrophoresis was carried out at 70 mA for 2 hr. The proteins were transferred onto nitrocellulose membranes and the membranes were incubated with 3% albumin-Tris-buffered saline, pH 7.5, overnight. The membranes were then incubated with anti-P-4502E1 immunoglobulin G (1:2000 dilution) for 2 hr. The antibody was raised in rabbits against the P-4502E1 purified from pyrazole-treated rats (Clejan and Cederbaum, 1990). The membranes were washed three times with 0.05% Tween 20 in Tris-buffered saline, followed by incubation with goat antirabbit immunoglobulin G conjugated with horseradish peroxidase (1:2500 dilution) for 1 hr. The membranes were rinsed three times with Tris-buffered saline and color was developed using a peroxidase color-developing reagent (Bio-Rad, Richmond, CA). Immunoreactive proteins were quantified using an LKB ultrascan XL laser densitometer (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Slot-blot analysis was carried out using 2 μg of total RNA, essentially as previously described (Winters and Cederbaum, 1992). The RNA was immobilized into nitrocellulose membrane filters by baking at 80°C in a vacuum oven for 2 hr and it was hybridized with a solution containing 50 μg/ml of salmon sperm DNA, 20% formamide, 5× SSPE (0.75M NaCl, 0.05M $NaH_2Po_4 \cdot H_2O$, 0.005M EDTA, pH 7.4), 2× Denhart's reagent and 0.1% sodium dodecyl sulfate at 47°C overnight. The membranes were then hybridized with a P-4502E1 oligonucleotide probe at 47°C overnight. The 51-mer oligonucleotide probe (Johansson *et al.*, 1988; Winters and Cederbaum, 1992) corresponds to the DNA sequence encoding amino acids 254 to 270 of P-4502E1, ccording to the published sequence of Song *et al.* (1986). The probe was synthesized on an Applied Biosystems 380B DNA synthesizer (Applied Biosystem Inc., Soster City, CA)and was labeled at the 5′ end with [γ-^{32}P]-ATP to a specific activity of 1.4×10^9 cpm/mg with polynucleotide kinase (Sambrook *et al.*, 1989). After several washings, the membranes were exposed to X-OMAT AR Kodak diagnostic X-ray film (Eastman Kodak, Rochester, NY) at −70°C for 3 days and analyzed by densitometry. After analysis, the membranes were placed in a boiling water bath for 10 min, cooled and rinsed to strip off the P-4502E1 oligonucleotide probe. The membranes were then hybridized with a 40-mer β-actin oligonucleotide probe (NEN Research Products, Boston, MA), which was labeled with [γ-^{32}P]-ATP and polynucleotide kinase to a final specific activity of 1.3×10^9 cpm/mg.

The results are the mean ± S.D., with the number of experiments indicated in the legends. Statistical analyses were carried out by Student's *t* test for unpaired data. Those results not indicated by statistical notation were not significant ($P > .05$).

Results

The growth rate of neonates from mothers who consumed ethanol during the gestational period and continued to consume ethanol during the neonatal period was less than that of controls (table 1). Under our reaction conditions, the total cytochrome P-450 was not spectrally detectable in fetal liver from either the controls or the ethanol-consuming mothers until birth. The content of cytochrome P-450 per milligram of microsomal protein increased during the neonatal period; there

TABLE 1

Body weight and cytochrome P-450 content of liver microsomes from rat fetus, neonate and mother

The fetuses on the 17th or 21st day of gestation, newborn neonates or pups on the 4th, 8th or 14th day after birth were weighed and analyzed for their hepatic content of cytochrome P-450. The results are from either four neonates or four pooled samples for the fetal livers. The mothers on the 17th or 21st day of gestation or 14 days after delivery were analyzed for their hepatic content of cytochrome P-450. The results are the mean of two (17th or 21st day gestation) or four rats.

Time Point	Body Weight		Hepatic Cytochrome P-450 Content	
	Control	Ethanol	Control	Ethanol
	g		*nmol/mg protein*	
Fetus and Neonate				
17th day of gestation	1 ± 0.1	0.9 ± 0.1	ND[a]	ND[a]
21st day of gestation	6.4 ± 0.3	5.4 ± 0.3	ND[a]	ND[a]
Day of birth	6.5 ± 0.5	5.3 ± 0.3	0.05 ± 0.03	0.04 ± 0.02
4 days neonatal	15.5 ± 0.5	9.5 ± 1.5*	0.10 ± 0.04	0.15 ± 0.07
8 days neonatal	19.5 ± 0.5	10.5 ± 0.5*	0.12 ± 0.04	0.16 ± 0.02
14 days neonatal	23.5 ± 0.5	14.5 ± 0.5*	0.24 ± 0.05	0.28 ± 0.04
Mother				
17th day of gestation			0.25	0.32
21st day of gestation			0.34	0.42
Day 14 after delivery			0.33 ± 0.07	0.55 ± 0.09*

[a] ND = not detectable.
* $P < .01$, ethanol treatment compared with control.

was no significant difference between the controls and neonates from mothers consuming ethanol (table 1). The total cytochrome P-450 content was elevated in the livers of the ethanol-consuming mothers. Chronic ethanol treatment has been shown to increase hepatic levels of cytochrome P-450 in adult rats (Lieber and DeCarli, 1970) and, in a guinea pig model, administration of two doses of ethanol per day resulted in a 65% increase in maternal hepatic cytochrome P-450 content (Card and Brien, 1989).

P-4502E1 protein levels were evaluated by western blot analysis. There was no detectable P-4502E1 in the fetal liver from either the controls or the ethanol-consuming mothers (fig. 1, table 2). P-4502E1 was detected shortly after birth and progressively increased during the neonatal period. The content of P-4502E1 was elevated in the hepatic microsomes from neonates of mothers who consumed ethanol. A slight increase was observed on the day of birth and an approximate 3-fold increase was found during the neonatal period (fig. 1, table 2). Hepatic microsomes from the ethanol-consuming mothers showed an approximate 2-fold induction of P-4502E1 (table 2).

The oxidation of effective substrates for P-4502E1, such as PNP or DMN, was determined to evaluate whether the increased P-4502E1 in neonatal livers from ethanol-consuming mothers was catalytically active. Neither substrate was oxidized in the fetal livers from controls or ethanol-consuming mothers (table 3) in agreement with the absence of P-4502E1. Low activity was found in hepatic microsomes on the day of birth; oxidation of PNP or DMN increased during the neonatal activity in agreement with the increasing content of P-4502E1. Oxidation of both substrates was elevated in hepatic microsomes from neonates of mothers who consumed ethanol during the gestational and neonatal periods; some increase could be observed on the day of birth and a 2- to 3-fold increase in oxidation of DMN or PNP was found throughout the neonatal period (table 3). Hepatic microsomes isolated from the ethanol-consuming mothers were more active than those from controls in oxidizing PNP and DMN, in agreement with their elevated levels of P-4502E1 protein.

These results indicate that consumption of ethanol during the gestational and neonatal period results in an increase in the content of P-4502E1 and oxidation of substrates for P-4502E1 in hepatic microsomes isolated from neonates and mothers. To evaluate possible mechanisms by which ethanol induced P-4502E1 in the neonates and the mothers, slot-blot analysis to assess the relative P-4502E1 mRNA levels were carried out. Low and comparable levels of P-4502E1 mRNA were present on the day of birth for controls and for pups from ethanol-consuming mothers (fig. 2, table 4). These levels of P-4502E1 mRNA were designated as 100 arbitrary units after the blots were scanned by densitometry. Very low, variable and almost background values for P-4502E1 mRNA were found in fetal liver. Levels of hepatic P-4502E1 mRNA increased during the neonatal period for control pups and pups from the ethanol-consuming mothers (fig. 2, table 4). By contrast with the results evaluating the content of P-4502E1 and associated catalytic activity, mRNA levels were not increased in the livers from pups of ethanol-consuming mothers. This lack of increase was noted whether the results were expressed as arbitrary units of P-4502E1 mRNA or as the P-4502E1 mRNA to actin mRNA ratio (table 4).

The mechanism whereby ethanol induces P-4502E1 in adult rats is complex (Koop and Tierney, 1990). Several reports suggest that the induction is not associated with elevated P-4502E1 mRNA levels (Song *et al.*, 1986; Peng *et al.* 1991; Yang *et al.*, 1990; Johansson *et al.*, 1988). However, some increase in steady-state P-4502E1 mRNA levels after chronic ethanol consumption has also been reported (Kubota *et al.*, 1988; Diehl *et al.*, 1991; Badger *et al.*, 1993). Although the content of P-4502E1 and oxidation of PNP and DMN were elevated in the hepatic microsomes of the pregnant rats consuming alcohol from day 9 of gestation and killed on day 17 or day 20 of gestation, P-4502E1 mRNA levels were similar in livers from the ethanol-consuming pregnant rats and controls (table 4). P-4502E1 mRNA levels were also similar in the livers of control rats and those of the rats consuming ethanol for a more prolonged period of time, *i.e.*, the last 13 days of gestation plus 14 days after delivery.

Discussion

Alcohol dehydrogenase activity is generally low in the fetal liver and the fetal blood concentration of ethanol is similar to the maternal blood alcohol levels (Card and Brien, 1989; Pikkarainen and Raiha, 1987; Card *et al.*, 1989; Abel and Greizer-

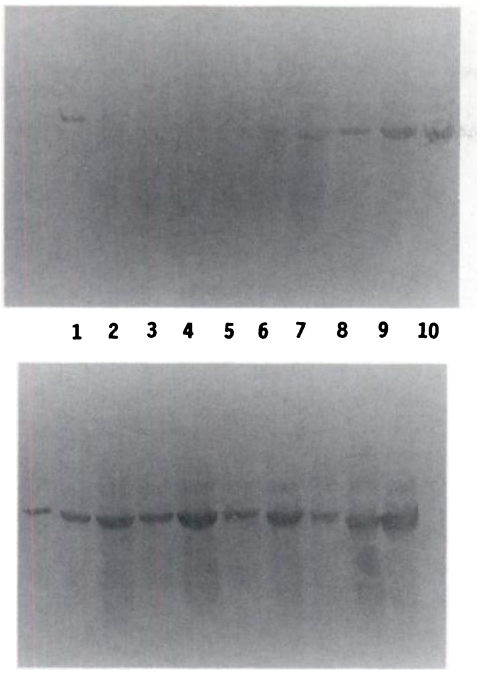

Fig. 1. Western blot of P-4502E1 in hepatic microsomes from rat fetus, neonate and mother. The experiments were carried out as described under Materials and Methods. Lane 1, 5 pmol of P-4502E1 purified from pyrazole-treated rats; 2, control fetus, 17th day of gestation; 3, fetus from ethanol-consuming mother, 17th day of gestation; 4, control fetus, 21st day of gestation; 5, ethanol-treated fetus, 21st day of gestation; 6, control, day of birth; 7, ethanol-treated pup, day of birth; 8, control, 4 days neonatal; 9, ethanol-treated pup, 4 days neonatal; 10, hepatic microsomes from control mother, 14 days after delivery; 11, 5 pmol of purified P-4502E1; 12, control, 8 days neonatal; 13, ethanol-treated pup, 8 days neonatal; 14, control, 14 days neonatal; 15, ethanol-treated pup, 14 days neonatal; 16, hepatic microsomes from control pregnant rat on 17th day of gestation; 17, hepatic microsomes from ethanol-consuming pregnant rat on 17th day of gestation; 18, hepatic microsomes from control pregnant rat on 21st day of gestation; 19, hepatic microsomes from ethanol-consuming pregnant rat on 21st day of gestation; 20, hepatic microsomes from ethanol-consuming mother 14 days after delivery (to be compared with control shown in lane 10). The results are from a typical experiment using one rat or a pooled sample from neonatal or fetal liver.

TABLE 2

Content of P-4502E1 in liver microsomes from rat fetus, neonate and mother

Western blot analysis was carried out as described under Materials and Methods using microsomes isolated from fetal liver on the 17th or 21st day of gestation or neonates as indicated. For fetuses and neonates, the results are the mean ± S.D. for either four neonates or four pooled samples of fetal liver. For mothers, microsomes were isolated from mothers on the 17th or 21st day of gestation or 14 days after delivery. The results are the mean of two (17th or 21st day gestation) or four rats.

Time Point	Content of Hepatic P-4502E1	
	Control	Ethanol
	Arbitrary units/mg microsomal protein	
Fetus and neonate		
17th day of gestation	ND[a]	ND[a]
21st day of gestation	ND[a]	ND[a]
Day of birth	35 ± 17	74 ± 28
4 days after birth	81 ± 52	227 ± 84*
8 days after birth	101 ± 46	240 ± 72*
14 days after birth	107 ± 46	320 ± 66**
Mother		
17th day of gestation	120	263
21st day of gestation	178	296
Day 14 after delivery	185 ± 22	337 ± 15**

[a] ND = not detectable.
* $P < .05$, ethanol treated compared with control.
** $P < .01$, ethanol treated compared with control.

stein, 1980). During pregnancy, the placenta is not a protective barrier to the alcohol consumed by the mother (Abel and Greizerstein, 1980). The distribution of ethanol within the fetus is related to the water content of various fetal tissues (Ho *et al.*, 1972). The low K_m aldehyde dehydrogenase of near-term fetal liver or in neonates was about the same as the adult hepatic activity (Card *et al.*, 1989). Chronic administration of ethanol to pregnant guinea pigs did not induce alcohol or aldehyde dehydrogenase activities in fetal (or maternal) liver (Card and Brien, 1989). It has been suggested that ethanol elimination is regulated by maternal hepatic alcohol dehydrogenase activity but the fetus was protected from ethanol-derived acetaldehyde by the presence of low K_m aldehyde dehydrogenase activity in fetal liver (Card and Brien, 1989; Card *et al.*, 1989; Pikkarainen and Raiha, 1987). The absence of P-4502E1 and the catalytic activities dependent on this enzyme in the fetal rat liver was confirmed in the present study. Shortly after birth, P-4502E1 mRNA, P-4502E1 protein and oxidation of PNP and DMN were detectable in the newborn pups and these parameters increased during the neonatal period. Transplacental induction of P-4502E1 by acetone has been reported (Hong *et al.*, 1987a); a similar induction by ethanol would be of importance in view of the ability of P-4502E1 to activate various carcinogens, drugs and solvents to reactive species (Koop, 1992; Yang *et al.*, 1990; Guengerich *et al.*, 1991; Lieber, 1988, 1990) and to generate reactive oxygen species (Gorsky *et al.*, 1984; Ekstrom and Ingelman-Sundberg, 1989; Cederbaum, 1989; Albano *et al.*, 1991). Ethanol and acetone may induce P-4502E1 by similar mechanisms, *i.e.*, stabilization of the protein against degradation (Song *et al.*, 1986, 1989; Koop and Tierney, 1990). However, consumption of ethanol by pregnant rats throughout the second half of the gestational period did not result in transplacental induction of P-4502E1 because neither P-4502E1 mRNA, protein or catalytic activity with PNP or DMN was detected in the fetal liver. Whether this reflects differences in available concentrations of ethanol or acetone or increased effectiveness of acetone as an inducer is not known. An oral model of ethanol intake, rather than injection of ethanol, was used to be of more physiological relevance to human studies. If induction of P-4502E1 by ethanol is mediated primarily at the post-transcriptional level (Song *et al.*, 1986; Peng *et al.*, 1991; Yang *et al.*, 1990; Johansson *et al.*, 1988), failure of transplacental induction of P-4502E1 by ethanol could reflect the lack of activation of the P-4502E1 gene in the fetal liver. It is not known whether other oral models (*e.g.*, intubation) of ethanol intake would have any effects on the induction of P-4502E1 in the fetal liver; however, we were able to detect induction by ethanol in the maternal liver under conditions in which no effects were found in the fetal liver.

The levels of P-4502E1 and oxidation of PNP or DMN were higher in the pups from the mothers consuming the ethanol-containing diet. Increases were found shortly after birth and continued throughout the neonatal period. The fact that increases were found shortly after birth and during the early neonatal period suggests that the ethanol present in the mother's milk was transferred to the newborn and was capable of inducing P-4502E1. It has been shown that ethanol is excreted

TABLE 3

Oxidation of PNP and DMN by hepatic microsomes from rat fetus, neonate and mother

For fetuses and neonates, oxidation of PNP or DMN was determined as described under Materials and Methods using microsomes isolated from fetal liver on the 17th or 21st day of gestation or neonates as indicated. The results are from either four neonates or four pooled samples. For mothers, hepatic microsomes were isolated from mothers on the 17th or 21st day of gestation or 14 days after delivery. The results are the mean of two (17th or 21st day gestation) or four rats.

Time Point	Oxidation of PNP		Oxidation of DMN	
	Control	Ethanol	Control	Ethanol
	nmol/min/mg protein		*nmol/min/mg protein*	
Fetus and neonate				
17th day of gestation	ND*	ND*	ND*	ND*
21st day of gestation	ND*	0.11 ± 0.08	ND*	0.03 ± 0.02
Day of birth	0.15 ± 0.15	0.27 ± 0.04	0.06 ± 0.03	0.18 ± 0.05*
4 days neonatal	0.46 ± 0.13	0.99 ± 0.35*	0.19 ± 0.08	0.33 ± 0.02*
8 days neonatal	0.54 ± 0.06	1.44 ± 0.25**	0.24 ± 0.03	0.59 ± 0.13**
14 days neonatal	0.80 ± 0.16	1.85 ± 0.44**	0.63 ± 0.17	1.10 ± 0.30*
Mother				
17th day of gestation	0.45	0.84	0.29	0.66
21st day of gestation	0.37	1.09	0.37	0.75
Day 14 after delivery	1.10 ± 0.39	2.69 ± 0.49*	0.26 ± 0.04	1.0 ± 0.03**

* ND = not detectable.
* $P < .05$, ethanol treated compared with control.
** $P < .01$, ethanol treated compared with control.

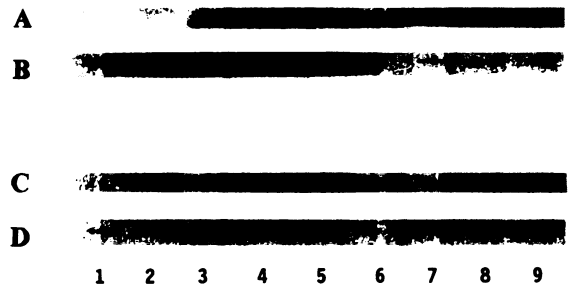

Fig. 2. Slot-blot analysis of P-4502E1 and actin mRNA in rat fetus, neonate and mother. The experiments were carried out as described under Materials and Methods. Slots A and B refer to P-4502E1 and actin mRNA, respectively, from ethanol-treated animals; slots C and D refer to P-4502E1 and actin mRNA, respectively, for controls. The lanes are identical for all slots. Lane 1, fetal liver, 17th day of gestation; 2, fetal liver, 21st day of gestation; 3, day of birth; 4, 4 days neonatal; 5, 8 days neonatal; 6, 14 days neonatal; 7, liver of pregnant rat on 17th day of gestation; 8, liver of pregnant rat on 21st day of gestation; 9, mother, 14 days after delivery.

TABLE 4

Content of P-4502E1 mRNA in liver from rat fetus, neonate and mother

Slot-blot analysis was carried out as described under Materials and Methods using 2 μg total RNA and oligonucleotide probes for the detection of P-4502E1 mRNA and actin mRNA. The results are expressed as either arbitrary units, setting the staining intensity values for day of birth samples for the control at 100 or as the ratio of P-4502E1 mRNA/actin mRNA, setting the values for day of birth samples for the control at 1.0.

Time Point	Hepatic P-4502E1 mRNA		Ratio of P-4502E1 mRNA/actin mRNA	
	Control	Ethanol	Control	Ethanol
	Arbitrary units			
Fetuses and neonates				
17th day of gestation	27 ± 20	30 ± 15	0.3 ± 0.2	0.2 ± 0.1
21st day of gestation	30 ± 15	30 ± 15	0.3 ± 0.2	0.3 ± 0.1
Day of birth	100 ± 5	104 ± 7	1.0 ± 0.1	1.0 ± 0.1
4 days neonatal	143 ± 38	183 ± 13	2.0 ± 0.1	1.8 ± 0.1
8 days neonatal	219 ± 31	197 ± 9	3.5 ± 0.1*	2.2 ± 0.1
14 days neonatal	197 ± 34	157 ± 11	1.9 ± 0.1	1.5 ± 0.1
Mother				
17th day of gestation	141	120	1.7	2.0
21st day of gestation	146	103	1.3	1.8
Day 14 after delivery	159 ± 8	130 ± 28	1.5 ± 0.1	2.1 ± 0.2

* $P < .05$, ethanol treated compared with control.

in breast milk in concentrations about 10% higher than that in the blood when absorption is complete (Kesaniemi, 1974; Lawton, 1985). The concentration of ethanol in breast milk has been found to be as high as 100 mg/dl (Binkeiwicz *et al.*, 1978) and, in humans, it has been calculated that the concentration of ethanol received by a nursing child could exceed 1 mM (Beattie, 1986; Holford, 1987). There are reports that excessive ethanol ingestion by a nursing mother can produce intoxication in the child (Holford, 1987) or produce a pseudo-Cushing syndrome (Binkeiwicz *et al.*, 1978). Because the induced P-4502E1 is catalytically active, the ability of ethanol, which is consumed by the mother, to be delivered to the newborn and to induce this enzyme immediately after birth and during the neonatal period could be of some toxicological significance if similar results occur in humans.

Regulation of P-4502E1 has been shown to be complex. Depending on the metabolic state, hormonal status or the chemical inducer, the concentration of P-4502E1 can be regulated by transcriptional and post-transcriptional events, including stabilization of P-4502E1 mRNA, increased translational efficiency of P-4502E1 mRNA and stabilization of P-4502E1 protein against degradation (Koop and Tierney, 1990). The mechanism of induction of P-4502E1 by ethanol in adult rats or rabbits has been suggested to be at the level of protein stabilization because ethanol did not increase P-4502E1 mRNA levels under conditions in which P-4502E1 protein was elevated (Song *et al.*, 1986; Peng *et al.*, 1991; Johansson *et al.*, 1988). Kubota *et al.* (1988) found that hamsters treated with ethanol displayed an increase in the translatable RNA corresponding to the increase in P-4502E1 protein. Increases in P-4502E1 mRNA levels after chronic ethanol consumption by rats has recently been reported (Diehl *et al.*, 1991; Badger *et al.*, 1993). In the neonates from ethanol-consuming mothers, the levels of P-4502E1 mRNA were not elevated compared with those in controls under conditions in which P-4502E1 protein and catalytic activity were increased. This suggests that the induction by ethanol in this liquid diet model is not at the transciptional level or does not involve stabilization of P-4502E1 mRNA. Previous results with pyrazole and 4-methylpyrazole (Wu and

Cederbaum, 1993) indicated that post-transcriptional regulation of P-4502E1 can occur almost immediately after birth. Although pyrazole and 4-methylpyrazole increased P-4502E1 content and catalytic activity, P-4502E1 mRNA levels were not altered in a regular Purina chow diet, similar to results with ethanol in the liquid diet. Similarly, induction of P-4502E2 in neonatal rabbits by imidazole was at the post-transcriptional level (Ding *et al.*, 1992). It is not known whether the induction by ethanol reflects an increased translation of P-4502E1 mRNA analogous to pyridine induction of P-4502E1 (Kim *et al.*, 1990) or stabilization of P-4502E1 at the protein level (Song *et al.*, 1989; Eliasson *et al.*, 1988).

The lower growth rate of neonates consuming ethanol may reflect the ability of ethanol to inhibit oxytocin release and suppress the milk ejection reflex (Fuchs *et al.*, 1967). Starvation increases P-4502E1 (Hong *et al.*, 1987a, 1987b; Johansson *et al.*, 1988) and it is possible that, because growth rates were lower in neonates from ethanol-consuming mothers, induction of P-4502E1 in the ethanol-treated pups may be the result, in part, of nutritional (and hormonal) interactions. Although a possible contributing factor, induction of P-4502E1 by fasting is associated with increases in P-4502E1 mRNA levels (Hong *et al.*, 1987a, 1987b; Johansson *et al.*, 1988). Because mRNA levels were not elevated under our reaction conditions, the induction of P-4502E1 in neonates from ethanol-consuming mothers cannot be explained solely by a fasting-like mechanism.

Induction of P-4502E1 occurred in the livers of pregnant rats consuming ethanol. Increases were found as few as 9 days after initiating the ethanol diet on day 9 of gestation and, as anticipated, were maintained after delivery as the mothers continued to consume ethanol. Induction of P-4502E1 and associated catalytic activity during pregnancy and in the mothers was a post-transcriptional event because P-4502E1 mRNA levels were not increased, analogous to induction by ethanol in adult nonpregnant rats.

In summary, these results indicate that, in an oral drinking model, transplacental induction of P-4502E1 by ethanol does not occur. However, continuous consumption of ethanol by pregnant rats up to the time of birth and early neonatal period results in induction of P-4502E1 in neonatal hepatic microsomes. The mechanism of induction appears to involve a post-transcriptional effect and suggests that ethanol derived from the mother's milk is available in sufficient amounts to the newborn rat to permit induction of P-4502E1.

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ERRATUM

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The Editor and Publisher regret the error which appeared in table 1 on page 1389. In the table, the listing for N-0923 should have been listed as the (−) isomer.