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INFLUENCES OF MATERNAL ETHANOL INTAKE ON MATERNAL AND PERINATAL HEPATIC HEME AND DRUG METABOLIZING ENZYMES IN RATS

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The effect of ethanol on maternal and neonatal hepatic heme and drug metabolizing systems was determined. Ethanol (16%, w/v) was administered orally as drinking solution to pregnant or lactating rats at different pre- and post-natal stages. The dams and pups were sacrificed on days 7, 14 and 21 after parturition, respectively.

Ethanol administration to lactating rats from just after birth caused an appreciable decrease in the maternal and neonatal body and liver weights. In addition, the activities of nicotinamide adenine dinucleotide phosphate-cytochrome c reductase, nicotinamide adenine dinucleotide-cytochrome b_5 reductase and heme oxygenase were significantly enhanced in the livers of neonates whose mothers were exposed to the ethanol during only first week of lactation, but those activities were not altered in the maternal livers.

However, no remarkable alterations were observed in the contents of cytochrome P-450 and b_5, and the activities of aminopyrine demethylase, aniline hydroxylase and δ-aminolevulinic acid synthetase in the livers of neonates from mothers who had received ethanol during lactation period or last week of gestation, although the activities of aminopyrine demethylase and aniline hydroxylase were enhanced significantly in lactating dams by ethanol consumption for 14 d after parturition.

Keywords —ethanol intake; lactation; neonate; fetal alcohol syndrome; cytochrome P-450; cytochrome b_5; NADPH-cytochrome c reductase; NADH-cytochrome b_5 reductase; heme oxygenase; δ-aminolevulinic acid synthetase

INTRODUCTION

Our previous study[1] demonstrated that ethanol intake in pregnant rats caused the decreases in the content of cytochrome b_5 and the activity of nicotinamide adenine dinucleotide (NADH)-cytochrome b_5 reductase in the liver of fetuses whose mothers were exposed to ethanol during from days 14 to 20 or throughout zero to 20 of gestation, and further tended to reduce the mean fetal body weight. Therefore, it would be of considerable interest to know if the maternal ethanol intake causes the significant influences on the hepatic heme and drug metabolizing activities in developing suckling.

Hence, the purpose of this study is to examine the ontogenesis of the activities in the hepatic heme and drug metabolizing enzymes in perinatal rats exposed to ethanol *via* either the transplacental route or *via* the milk.

MATERIALS AND METHODS

Chemicals —Hemin was obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. δ-Aminolevulinic acid (ALA) hydrochloride was obtained from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan. Cytochrome c was purchased

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from Sigma Chemical Company, St. Louis, USA. Nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate, glucose 6-phospate disodium salt, and glucose 6-phosphate dehydrogenase were obtained from Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan. Ethanol was used following purification by redistillation. Other chemicals of reagent grade were obtained from commercial sources and used without further purification.

Animals — Wistar rats weighing 250–300 g were used in this experiment. For a one-week period before mating, all animals were maintained on commercial rat chow (Clea CE-2, Nippon Clea Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. Two females with one male of the same age were placed in same breeding cage overnight. The day on which sperms were found in a vaginal smears was marked as day 0 of gestation. Female rats on day 0 of gestation were randomly divided into three treatment groups and one control group, and housed individually in each cage. The first group received ethanol in 16 g per 100ml water as drinking solution only during days 14 to 21 of gestation *ad libitum*, and pregnant females were allowed to give birth normally. The second group was allowed to deliver at first, and then received ethanol *ad libitum* from day first to 7, 14 and 21 after parturition respectively. The third group received ethanol throughout from days 14 of gestation to 7, 14 and 21 after parturition respectively. Ethanol treated groups were given food *ad libitum*, and control group received food and water *ad libitum* during this experiment. The neonates and dams in untreated or treated groups of ethanol were sacrificed on day 7, 14 and 21 postpartum respectively, by decapitation or cervical dislocation.

Assay Procedures — The livers of dams were perfused *in situ* with a cold 0.9% NaCl solution, and then removed and saved for preparation of microsomes. The neonatal livers were removed and livers of littermate were pooled for preparation of microsomes. Microsomes were prepared by the method described previously.[2] Protein concentration was determined according to Lowry *et al.*[3] using bovine serum albumin as a standard. ALA synthetase activity of liver homogenates was determined by the method of Marver *et al.*[4] Heme oxygenase activity of microsomes was measured by the method of Maines and Kappas.[5] Cytochrome P-450 and b_5 contents were estimated as described by Omura and Sato.[6] The activities of NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase were determined by the method of Omura and Takesue[7] and by that of Takesue and Omura,[8] respectively. Aminopyrine N-demethylase activity was determined by Nash reaction[9] for formaldehyde produced from the oxidative demethylation reaction. Aniline hydroxylase activity was determined according to Imai *et al.*[10]

RESULTS

The influences of ethanol administration to pregnant and lactating female rats on the maternal and neonatal body and liver weights and litter size at day 7, 14 and 21 postpartum are summarized in Table I (a, b and c).

All pregnant females delivered between days 21 and 22. The litter size from the controls and each ethanol-treated dams was about 13 neonates per rat except the newborns at day 21 postpartum whose mothers recieved ethanol throughout pre- and post-partum periods. Two or three neonates from control group died within 14 d after parturition. However, no marked difference was observed in the neonatal deaths between the controls and each ethanol treated group, as shown in Table I (a, b and c). In ethanol treated first group, there was no difference in maternal and their neonatal body and liver weights during this experimental period. On the contrary, maternal and neonatal body and liver weights in ethanol treated second group markedly reduced on day 7, 14 and 21 postpartum, respectively. In the third group which had received ethanol throughout pre- and post-natal period, these maternal weights were also lower on day 7 and 14 postpartum, and

TABLE I. *Effects of Maternal Ethanol Intake on Neonatal and Maternal Body and Liver Weights on Day 7, 14 and 21 after Parturition*

		Ethanol administered on days		
a: day 7 after parturition	Control	14—21 of gestation	1—7 after birth	14 of gestation to 7 after birth
Number of litters	7	5	4	4
Number of newborns	73	50	42	34
Number of newborns per litter	10±1	10±2	11±2	9±2
Neonatal body weight (g)	12.71±1.02	11.20±0.97	10.00±0.41 a)	10.50±0.50
Noenatal liver weight (g)	0.34±0.03	0.29±0.03	0.25±0.01 a)	0.29±0.03
(g/g of b.w.)	0.027±0.001	0.026±0.001	0.025±0.001	0.028±0.003
Maternal body weight (g)	283±11	282±8	243±5 b)	244±4 b)
Maternal liver weight (g)	11.85±0.63	12.57±0.47	8.97±1.04 a)	8.28±0.46 b)
(g/100 g b.w.)	4.19±0.17	4.48±0.28	3.68±0.36	3.39±0.15 b)
Daily ethanol intake (g/mother)	—	2.97±0.44	3.32±0.72	2.80±0.13
b: day 14 after parturition	Control	14—21 of gestation	1—14 after birth	14 of gestation to 14 after birth
Number of litters	4	4	4	5
Number of newborns	41	38	45	55
Number of newborns per litter	10±2	10±2	11±1	11±1
Neonatal body weight (g)	23.25±1.93	18.00±3.19	17.25±0.95 a)	16.20±3.23
Noenatal liver weight (g)	0.69±0.13	0.61±0.09	0.45±0.03 b)	0.43±0.12
(g/g of b.w.)	0.029±0.001	0.034±0.002	0.026±0.001	0.026±0.002
Maternal body weight (g)	299±13	314±10	234±5 b)	248±8 a)
Maternal liver weight (g)	13.10±0.92	15.17±1.44	9.11±0.26 b)	10.50±1.12 a)
(g/100 g b.w.)	4.37±0.15	4.83±0.42	3.89±0.04 a)	4.19±0.19
Daily ethanol intake (g/mother)	—	3.28±0.30	3.66±0.24	3.94±0.68
c: day 21 after parturition	Control	14—21 of gestation	1—21 after birth	14 of gestation to 21 after birth
Number of litters	5	4	4	4
Number of newborns	50	49	48	25
Number of newborns per litter	10±1	12±1	12±1	6±1 a)
Neonatal body weight (g)	34.00±2.02	35.00±3.54	21.75±3.86 a)	37.25±5.62
Noenatal liver weight (g)	1.21±0.09	1.21±0.15	0.69±0.07 b)	1.34±0.24
(g/g of b.w.)	0.033±0.001	0.035±0.001	0.033±0.003	0.036±0.001
Maternal body weight (g)	326±4	293±27	265±15 b)	307±18
Maternal liver weight (g)	15.64±0.90	13.58±1.38	10.99±0.68 b)	12.65±1.04
(g/100 g b.w.)	4.80±0.25	4.63±0.08	4.15±0.08 a)	4.11±0.19
Daily ethanol intake (g/mother)	—	3.55±0.19	4.07±0.41	4.44±0.67

Animals were received ethanol as drinking solution on days 14—21 of gestation, 1—7 or 1—14 or 1—21 after birth and 14 of gestation to 7 or 14 or 21 after birth, respectively, and were sacrificed on day 7 or 14 or 21 after parturition together with their newborns. Each value represents the mean ± S.E. of 4 to 7 rats or 4 to 7 l. Significantly different from corresponding mean of control.
a) $p < 0.05$ or b) $p < 0.01$.

neonatal body and liver weights tended to be lower when compared with those of untreated controls. However, no marked difference was observed in the mean daily consumption of ethanol among above three experimental groups.

Table II (a, b and c) shows the effects of treatments with ethanol at various intervals to pregnant and lactating rats on heme metabolism and drug metabolizing systems in neonatal livers.

For the ethanol treated first and third groups, there were no marked changes in the contents of cytochrome P-450 and b_5 and in the enzyme activities of heme and drug metabolizing systems in the neonates sacrificed at 7 d after birth. The neonates in the second group, namely whose mothers had received ethanol only after parturition, showed the increases in the activities of heme oxygenase (182% of control), NADPH-cytochrome c reductase (132% of control) and NADH-cytochrome b_5 reductase (132% of control) when compared with those of neonates from untreated control mothers. On the other hand, no remarkable alterations were observed upon above measured parameters in the newborns at days 14 and 21 after delivery except heme oxygenase activity in neonates of the second group at 14 d after parturition.

Alterations in hepatic heme and drug metabolizing enzymes were also determined in the dams. The summarized data are presented in Table III (a, b and c). Significant increases in the activities of aniline hydroxylase and aminopyrine demethylase were observed in the livers from dams who had received ethanol after parturition or throughout from late stage of pregnancy to postpartum except the dams who were sacrificed at 21 d after delivery, but in the second group the enhanced activity in the aniline hydroxylase was still observed. Cytochrome P-450 content on day 7 postpartum was markedly increased in the livers of mothers in ethanol treated third group, though heme metabolic enzyme activities were not affected in each ethanol treated dams when compared to values obtained with untreated control dams.

DISCUSSION

It has been reported that maternal ethanol consumption during pregnancy in humans seriously effected on the developing fetuses and produced various clinical abnormalities, which is termed as fetal alcohol syndrome.[11-18] In the experimental animals it was found that maternal ethanol intake during gestation markedly decreased the size and number of progeny produced.[19,20] In addition, we noted that ethanol intake to pregnant rats reduced the hepatic microsomal NADH-cytochrome b_5 reductase activity in fetuses.[1] Therefore, this study is designed to follow the effects of maternal ethanol intake on the heme metabolism and the drug metabolizing systems in the sucking neonates.

The treatments with ethanol to pregnant rats are as follows: first, pregnant females received ethanol only in the rate stage of gestation; namely this treatment is based on the assumption that a few mothers stopped the ethanol intake by good chance of childbirth, secondly received ethanol after parturition in order to examine the influences of lactation and thirdly received that throughout from the last week of pregnancy to ablactation period in order to investigate the effects of maternal ethanol abuse in the perinatal period, respectively. As shown in Table I (a, b and c), there is no marked difference in ethanol consumption among these three experimental groups. However, a slight increase in ethanol intake (but not significant) was noted in the groups which were sacrificed at 21 d after birth. This suggests that certain newborns may drink up ethanol with their mother just before ablactation period.

The significant reduction of neonatal body and liver weights was observed in the newborns from mothers who had received ethanol only after parturition. When liver weight calculated on gram body weight, however, no significant change in the liver weight was observed. Consequently, the observed reduction in liver weight seems to depend on the lower gain of body weight. Previous studies[1,2] from this laboratory have shown that when ethanol administered

TABLE II. *Alterations in Hepatic Enzyme Activities of Heme and Drug Metabolizing Systems in Neonatal Rats after Maternal Ethanol Intake during Gestation and Lactation*

a: day 7 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–7 after birth	14 of gestation to 7 after birth
Number of litters	7	5	4	4
Cytochrome P-450[a]	0.41 ±0.02	0.39 ±0.06	0.49 ±0.04	0.38 ±0.04
Cytochrome b_5[a]	0.18 ±0.01	0.21 ±0.02	0.21 ±0.02	0.17 ±0.01
NADPH-cytochrome c reductase[b]	12.56 ±0.27	16.18 ±1.86	16.58 ±0.75[f]	14.29 ±0.49
NADH-cytochrome b_5 reductase[c]	0.97 ±0.06	1.11 ±0.05	1.29 ±0.03[f]	0.99 ±0.06
Aminopyrine demethylase[b]	0.42 ±0.09	0.49 ±0.24	0.47 ±0.04	0.33 ±0.09
Aniline hydroxylase[b]	0.51 ±0.03	0.55 ±0.02	0.61 ±0.05	0.52 ±0.03
δ-Aminolevulinic acid synthetase[d]	29.8 ±1.6	–	40.1 ±5.0	–
Heme oxygenase[e]	3.35 ±0.37	3.66 ±0.32	4.92 ±0.18[f]	4.03 ±0.48

b: day 14 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–14 after birth	14 of gestation to 14 after birth
Number of litters	4	4	4	5
Cytochrome P-450[a]	0.54 ±0.06	0.33 ±0.08	0.39 ±0.03	0.45 ±0.07
Cytochrome b_5[a]	0.30 ±0.01	0.21 ±0.04	0.29 ±0.01	0.28 ±0.02
NADPH-cytochrome c reductase[b]	15.38 ±1.07	18.18 ±1.69	16.14 ±0.37	16.07 ±0.89
NADH-cytochrome b_5 reductase[c]	1.21 ±0.08	0.90 ±0.10	1.07 ±0.06	2.13 ±0.42
Aminopyrine demethylase[b]	0.79 ±0.08	0.77 ±0.17	0.63 ±0.07	0.63 ±0.15
Aniline hydroxylase[b]	0.79 ±0.05	0.65 ±0.04	0.72 ±0.05	0.78 ±0.05
δ-Aminolevulinic acid synthetase[d]	41.5 ±6.2	–	36.6 ±3.3	–
Heme oxygenase[e]	2.22 ±0.29	3.12 ±0.46	3.55 ±0.33	3.18 ±0.39

c: day 21 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–21 after birth	14 of gestation to 21 after birth
Number of litters	5	4	4	4
Cytochrome P-450[a]	0.57 ±0.05	0.59 ±0.07	0.55 ±0.08	0.63 ±0.03
Cytochrome b_5[a]	0.30 ±0.02	0.33 ±0.01	0.35 ±0.03	0.32 ±0.01
NADPH-cytochrome c reductase[b]	23.85 ±1.31	25.27 ±0.95	24.21 ±2.42	24.31 ±1.34
NADH-cytochrome b_5 reductase[c]	1.49 ±0.05	1.47 ±0.08	1.27 ±0.16	1.46 ±0.10
Aminopyrine demethylase[b]	1.07 ±0.13	1.43 ±0.18	1.20 ±0.16	1.33 ±0.22
Aniline hydroxylase[b]	0.97 ±0.31	0.97 ±0.08	1.29 ±0.22	1.03 ±0.09
δ-Aminolevulinic acid synthetase[d]	55.9 ±6.0	–	58.4 ±0.8	–
Heme oxygenase[e]	2.05 ±0.39	1.73 ±0.17	1.93 ±0.40	1.73 ±0.26

Conditions are described in Table I. Newborns were sacrificed and livers from neonates of each litter were pooled. Each value represents the mean ± S.E. of 4 to 7 l.
a) nmol/mg protein; b) nmol/mg protein/min; c) μmol/mg protein/min; d) nmol δ-ALA/g liver/h; e) nmol bilirubin/mg protein/h. Significantly different from corresponding mean of control, f) $p < 0.01$.

chronically to rats, the lower gain of body weight was observed. Thus this weight gain in neonates might be due to the direct effect of ethanol because ethanol easily can be transmitted

TABLE III. *Postpartum Changes in Hepatic Enzyme Activities of Heme and Drug Metabolizing Systems in Lactating Rats Receiving Ethanol during Perinatal Period*

a: day 7 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–7 after birth	14 of gestation to 7 after birth
Number of litters	7	5	4	4
Cytochrome P-450[a]	0.64±0.04	0.69±0.06	0.81±0.12	0.92±0.08[f]
Cytochrome b_5[a]	0.34±0.01	0.43±0.04	0.43±0.03	0.40±0.03
NADPH-cytochrome c reductase[b]	25.39±1.10	29.82±2.79	28.06±0.53	26.49±1.98
NADH-cytochrome b_5 reductase[c]	3.36±0.19	3.96±0.17	3.65±0.27	2.85±0.19
Aminopyrine demethylase[b]	1.76±0.12	1.54±0.31	2.50±0.25[f]	2.77±0.26[f]
Aniline hydroxylase[b]	0.48±0.04	0.52±0.04	1.61±0.33[g]	1.98±0.33[g]
δ-Aminolevulinic acid synthetase[d]	55.3±3.8	–	63.1±2.3	–
Heme oxygenase[e]	0.88±0.09	0.76±0.22	0.72±0.09	0.70±0.08

b: day 14 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–14 after birth	14 of gestation to 14 after birth
Number of litters	4	4	4	5
Cytochrome P-450[a]	0.65±0.05	0.56±0.05	0.74±0.06	0.86±0.09
Cytochrome b_5[a]	0.40±0.02	0.37±0.03	0.39±0.02	0.41±0.04
NADPH-cytochrome c reductase[b]	27.19±2.73	25.07±1.59	26.81±2.16	27.77±1.78
NADH-cytochrome b_5 reductase[c]	3.76±0.36	3.82±0.19	3.16±0.29	3.87±0.21
Aminopyrine demethylase[b]	1.75±0.32	1.57±0.11	2.59±0.15[f]	2.72±0.33[f]
Aniline hydroxylase[b]	0.55±0.03	0.50±0.03	1.83±0.29[g]	2.24±0.10[g]
δ-Aminolevulinic acid synthetase[d]	53.5±3.6	–	72.8±7.7	–
Heme oxygenase[e]	0.84±0.20	0.56±0.14	0.66±0.04	0.85±0.13

c: day 21 after parturition	Control	Ethanol administered on days: 14–21 of gestation	1–21 after birth	14 of gestation to 21 after birth
Number of litters	5	4	4	4
Cytochrome P-450[a]	0.66±0.04	0.53±0.08	0.72±0.05	0.69±0.07
Cytochrome b_5[a]	0.38±0.02	0.32±0.04	0.37±0.03	0.34±0.04
NADPH-cytochrome c reductase[b]	28.24±1.10	31.89±4.76	28.58±0.77	29.13±2.94
NADH-cytochrome b_5 reductase[c]	3.86±0.45	3.94±0.59	3.57±0.22	3.86±0.41
Aminopyrine demethylase[b]	1.60±0.35	1.59±0.12	2.58±0.31	1.61±0.21
Aniline hydroxylase[b]	0.55±0.03	0.36±0.04	1.26±0.23[f]	0.87±0.22
δ-Aminolevulinic acid synthetase[d]	53.0±3.1	–	55.5±6.6	–
Heme oxygenase[e]	0.79±0.37	0.92±0.23	0.99±0.21	0.89±0.20

Conditions were as described in Table I. Each value represents the mean ± S.E. of 4 to 7 dams. a) nmol/mg protein; b) nmol/mg protein/min; c) μmol/mg protein/min; d) nmol δ-ALA/g liver/h; e) nmol bilirubin/mg protein/h. Significantly different from corresponding mean of control, f) $p < 0.05$ or g) $p < 0.01$.

through maternal milk to the sucking rats.

In the third group maternal ethanol intake in the perinatal period caused no significant alterations in neonatal body and liver weights. This

TABLE IV. *Hepatic Cytochrome Contents and Enzyme Activities in the Fetuses on Day 20 of Gestation and in the Developing Neonates on Days 7, 14 and 21 after Parturition*

	Fetus[a] gestation day 20	Neonate		
		Lactation day 7	Lactation day 14	Lactation day 21
Number of litters	6	7	4	5
Cytochrome P-450[b]	0.02±0.01	0.41±0.02	0.54±0.06	0.57±0.05
Cytochrome b_5[b]	0.06±0.00	0.18±0.01	0.30±0.01	0.30±0.02
NADPH-cytochrome c reductase[c]	5.01±0.61	12.56±0.27	15.38±1.07	23.85±1.31
NADH-cytochrome b_5 reductase[d]	0.63±0.05	0.97±0.06	1.21±0.08	1.49±0.05
Aminopyrine demethylase[c]	–	0.42±0.09	0.79±0.08	1.07±0.13
Aniline hydroxylase[c]	–	0.51±0.03	0.79±0.05	0.97±0.31
δ-Aminolevulinic acid synthetase[e]	–	29.8±1.6	41.5±6.2	55.9±6.0
Heme oxygenase[f]	2.24±0.34	3.35±0.37	2.22±0.29	2.05±0.39

Fetuses and neonates were sacrificed on day 20 of gestation and on days 7, 14 and 21 after parturition, respectively. The livers from the fetuses and the neonates of each litters were pooled.

Each value represents the mean ± S.E. of 4 to 7 l. a) cited for reference 1; b) nmol/mg protein; c) nmol/mg protein/min; d) μmol/mg protein/min; e) nmol δ-ALA/g liver/h; f) nmol bilirubin/mg protein/h.

reason is not known, but it may be due to decreases in the ethanol content in milk as a result of adaptive increases in the maternal liver to the ethanol.

Numerous investigators[21-24] reported that drug metabolizing enzyme activities in fetuses are negligible or very small, and these activities increase postpartum and then approach at values of adult levels at the end of lactation period. In the developing neonates from untreated mothers, in this study, aminopyrine demethylase and aniline hydroxylase activities showed marked increase with an increase in cytochrome P-450 and b_5 contents and NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase activities. This summarized result is presented in Table IV, and its result agreed with other observations.[21-24]

Although we previously observed[1] that maternal ethanol intake decreased the NADH-cytochrome b_5 reductase activity in fetal livers on day 20 of gestation, the suckling rat livers at 7 d after birth showed significant increases in the activities of NADH-cytochrome b_5 reductase and NADPH-cytochrome c reductase in this study. This observed difference in the NADH-cytochrome b_5 reductase activity in the fetal and neonatal livers is difficult to explain. However, this may partly be associated with the difference in the ability of metabolism to ethanol. Ethanol is metabolized in the liver mainly by the enzyme alcohol dehydrogenase (ADH), which requires NAD as cofactor. The activity of ADH increases markedly around birth and the postnatal increase is rather linear. Consequently NADH production from ethanol metabolism in neonates is more greater than that in fetuses. This difference in the hepatic intracellular NADH redox state may exert some influences on NADH-linked enzyme system. Further the difference in the above enzyme activity was also observed in the groups between the second and the third at 7 d after birth. This may be caused by the difference in the ethanol content between two groups, that is, ethanol content in the second group seems higher than that in the third as ADH activity is similar. Becuase maternal hepatic cytochrome P-450 content in the third group is more higher than that in the second group, as shown in Table III (a). This fact may

have some relation on the metabolism and content of ethanol in mothers and in their neonates.

In addition, in the second group the cytochrome P-450 content and aniline hydroxylase activity in the neonatal liver at 7 d after birth showed the tendency to be increased (120% of control, respectively) by the maternal ethanol intake.

These effects appear to result from the postnatal exposure to ethanol in the milk, because it was reported that acute or chronic treatment with ethanol induced the hepatic drug metabolizing systems.[2,25-29] However, in the newborns at 14 and 21 d after parturition exposed to ethanol *via* the maternal milk, no distinct difference in all measured parameters were observed. This may result from adaptation to the longer period of ethanol exposure. Further study on the determination of ethanol in blood and breast milk of lactating mothers will be needed. However, above findings suggest that the inducing effect of ethanol *via* the milk is relatively low when compared to the inducing ability of phenobarbital *via* the maternal milk.[21-24]

ALA synthetase activity, the first and rate-limiting enzyme of heme synthesis, was not significantly altered in the suckling neonates even at 7 d and naturally 14 or 21 d after birth by maternal ethanol intake. On the other hand, the heme oxygenase activity, the first and rate-limiting enzyme of heme degradation, appreciably increased in the suckling rats on day 7 or 14 postpartum. However, continuous administration of ethanol to lactating mothers did not produce the change in the activities of heme metabolizing enzymes in the livers of mothers. This observed difference on heme oxygenase activity between neonates and their mothers who received ethanol still remains in the question. Furthermore, it is interesting that heme oxygenase activity in suckling rats was higher than that in their mothers when calculated on mg microsomal protein. But this high activity was decreased with the lapse of time. The high activity of heme oxygenase in neonates may originally be necessary to treat an inordinate physiological substrate-heme, because the neonates just after birth require a rapid metabolism of red blood cell due to an age of transition from fetal respiration to neonatal respiration. In this study, heme oxygenase activity in early and middle stages of lactation period was enhanced by maternal ethanol intake. It is unknown whether or not ethanol initially induce a perturbation of substrate-heme and thereby stimulate heme oxygenase. However, there is no remarkable effect of ethanol on heme oxygenase in the livers of lactating mothers and also of late stage of suckling rats. Papara-Nicholson and Telford[30] reported that guinea pig given ethanol throughout their pregnancies, produced offspring with abnormally low birth weights, poor locomotion and anemia, namely hemoglobin content in the offspring is 70 to 80% of normal. In addition, it is well known that acute immoderate drinking in man leads to an adverse effects such as hemorrhagic erosion of gastrointestinal mucosa, gatrointestinal hemorrhage, gastric ulcer and hematemesis. From these results and our findings, the enhanced heme oxygenase activity in early and middle stages of lactation period appears to be due to the increased substrate-heme by exposure to ethanol *via* the maternal milk. In general, newborns have much less activity in glucuronyltransferase than that in adult. Consequently maternal ethanol intake in the perinatal period may exacerbate the bilirubinemia in neonates with an increase of heme oxygenase activity. However, this enzyme activity in neonates will indeed require further investigation.

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