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ONTOGENY OF THE ACTIVITY OF ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASES IN THE LIVER AND PLACENTA OF THE GUINEA PIG

SHARON E. CARD, SEAN F. TOMPKINS and JAMES F. BRIEN*
Department of Pharmacology and Toxicology, Faculty of Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6

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Abstract—The objectives of this study were to elucidate the ontogeny of the activity of alcohol dehydrogenase (ADH), low K_m aldehyde dehydrogenase (ALDH) and high K_m ALDH in the liver and placenta of the guinea pig, and to determine the relationship between the relative activity of each enzyme in the guinea pig maternal–placental–fetal unit and the disposition of ethanol and its proximate metabolite, acetaldehyde. The enzyme activities were determined in maternal liver, fetal liver, and placenta of the guinea pig at 34, 50, 60 and 65 days of gestation (term, about 66 days), in the liver of the 2-day-old neonate, and in adult liver. There was low ADH activity in fetal liver and placenta throughout gestation and in neonatal liver. The fetal liver low K_m ALDH activity increased progressively and, at 60 days of gestation, was similar to adult liver activity, as was also the case for neonatal liver enzyme activity. Placental low K_m ALDH activity was less than adult liver activity throughout gestation. Fetal hepatic high K_m ALDH activity increased during gestation, but was less than adult liver activity, as was also the case for neonatal liver enzyme activity. Placental high K_m ALDH activity was low throughout gestation. For oral administration of 0.5 g ethanol/kg maternal body weight to pregnant guinea pigs at mid-gestation (34 days), the maternal blood and fetal body ethanol concentration–time curves were similar. Acetaldehyde was measurable in maternal blood and fetal body at similar concentrations, which were 100- to 1000-fold less than the respective ethanol concentrations. The major difference in the disposition of ethanol and acetaldehyde at near-term pregnancy, compared with mid-gestation, was the lack of measurable acetaldehyde in fetal blood. These results indicate that the guinea pig fetus throughout gestation has virtually no capacity to oxidize ethanol, and its duration of exposure to ethanol is regulated by maternal hepatic ADH-catalyzed biotransformation of ethanol. The fetus, however, appears to have increasing low K_m ALDH-dependent capacity to oxidize ethanol-derived acetaldehyde during development, and would appear to be increasingly protected from exposure to acetaldehyde as gestation progresses.

Ethanol ingestion during pregnancy can produce a wide spectrum of effects on the conceptus, ranging from teratogenic effects (the fetal alcohol syndrome) [1, 2] to suppression of fetal breathing-like movements [3, 4], electrocortical activity [4] and cerebral oxidative metabolism [5]. The determination of the disposition of ethanol is important in the elucidation of the mechanisms of the embryo–fetal effects of ethanol, especially in view of the finding that the incidence of teratogenic effects appears to be more closely related to the blood ethanol concentration than to the absolute amount of ethanol consumed [6, 7]. The pharmacokinetics of ethanol have been elucidated for near-term pregnancy only, and include similar maternal blood and fetal blood ethanol concentration–time curves and differential disposition of ethanol in amniotic fluid relative to maternal blood and fetal blood leading to ethanol accumulation in the amniotic fluid compartment [8–15].

Acetaldehyde, the proximate metabolite of ethanol, has intrinsic pharmacological activity [16]. There is evidence that supports [17, 18] and refutes [6, 19] the involvement of acetaldehyde in the embryo–fetal toxicity of maternal ethanol ingestion. We have measured ethanol-derived acetaldehyde in maternal blood and fetal blood of near-term pregnant animals at concentrations about 1000-fold less than the respective ethanol concentrations [11–15]. Furthermore, the acetaldehyde concentration in fetal blood was less than that in maternal blood.

The liver is the major site for the oxidation of ethanol to acetaldehyde and the subsequent oxidation of acetaldehyde to acetate [20], and these two reactions are catalyzed primarily by alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1, ADH) and aldehyde dehydrogenase (aldehyde:NAD oxidoreductase, EC 1.2.1.3, ALDH), respectively. For the near-term pregnant ewe, the K_m for *in vivo* maternal hepatic biotransformation of ethanol has been shown to be similar to the K_m for ethanol of adult hepatic ADH activity determined *in vitro* [11, 14, 15]. These data, together with the very low ADH activity in near-term fetal liver and placenta relative to maternal liver [12, 21, 22], indicate that maternal hepatic ADH-catalyzed biotransformation of ethanol regulates the overall elimination of ethanol from the maternal and fetal compartments during late gestation. The observation that the maternal blood acetaldehyde concentration is about 1000-fold less (μM range) than the ethanol concentration (mM range) [11–15] indicates that, during near-term pregnancy, there is

* Correspondence: Dr James F. Brien, Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

appreciable acetaldehyde-oxidizing capacity in the maternal liver involving low K_m (μM acetaldehyde) ALDH. Furthermore, the lower acetaldehyde concentration in fetal blood compared with maternal blood and the similar activity of low K_m ALDH in fetal liver and maternal liver during near-term pregnancy [12, 22] indicate that there is appreciable acetaldehyde-oxidizing capacity in the maternal–fetal unit during late gestation, which limits the exposure of the fetus to ethanol-derived acetaldehyde. The relationship between the pharmacokinetics of ethanol and its proximate metabolite, acetaldehyde, and the activities of ADH and low K_m ALDH at earlier gestational ages is not known.

The objectives of the present study were to elucidate the ontogeny of ADH, low K_m ALDH and high K_m ALDH activities in the liver and placenta, focusing on several mid-to-late gestational ages, and to determine the relationship between the relative activity of each enzyme in the maternal–placental–fetal unit and the disposition of ethanol and its proximate metabolite, acetaldehyde. The guinea pig was selected for this study as its placenta has some characteristics similar to those of the human placenta [23]; it is more developed biochemically, morphologically and functionally at birth than the rat [23]; it has a long trimester-type gestational period (59–72 days; mean, 66 days) [24]; and the pharmacokinetics of ethanol are similar for the near-term pregnant guinea pig [12, 13] and pregnant human at term [25].

MATERIALS AND METHODS

Chemicals and solutions. NAD^+, NADH, pyrazole, rotenone, sodium deoxycholate, Tris–HCl and tetrasodium EDTA were obtained from the Sigma Chemical Co. (St Louis, MO). Acetaldehyde (99% pure) was obtained from the Aldrich Chemical Co. (Milwaukee, WI), and absolute ethanol was purchased from Consolidated Alcohols Ltd (Toronto, Ontario, Canada). All other chemicals were at least reagent grade and were obtained from a variety of commercial sources. All chemical solutions and buffers were prepared using deionized water, except for the rotenone solution, which was prepared in methanol.

Experimental animals. Nulliparous female Hartley-strain guinea pigs, 450–600 g body weight (Charles River Canada Inc., St-Constant, Quebec, Canada), were bred with male Hartley-strain guinea pigs. Day 0 of pregnancy was established by the observation of a vaginal plug and/or the last day of full vaginal-membrane opening. Fetal gestational age was confirmed on the last day of experimentation by comparing the mean weight of the litter with the fetal age/weight curves of Draper [26]. The animals were housed in groups of three or four in stainless-steel wire cages with a 12-hr light/12-hr dark cycle with lights on at 7:00 a.m. Purina Guinea Pig Chow 5025® and water were provided *ad lib.* The animals were monitored at least every other day for body weight and general health. All animals were cared for according to the principles and guidelines of the Canadian Council on Animal Care.

In vitro *determination of the activity of ADH and ALDH in liver and placenta.* Pregnant guinea pigs at the following mean gestational ages: 34 days (range, 32–35 days; N = 6), 50 days (range, 49–51 days; N = 5), 60 days (range, 58–60 days; N = 6) and 65 days (range, 64–65 days; N = 6), 2-day-old neonatal animals (N = 5) and adult (>75 days of age) male (N = 3) and female (N = 5) guinea pigs were studied. Each animal was killed by decapitation. For each pregnant animal, the individual placentae and fetal livers and the maternal liver were perfused with ice-cold buffered saline (50 mM KH_2PO_4, 150 mM NaCl, pH 7.2), and then placed in ice-cold sucrose buffer (0.25 M sucrose, 5 mM Tris–HCl, 0.5 mM EDTA, pH7.2). The liver of each neonatal and adult animal was processed by the same procedure. Pooled fetal livers and pooled placentae of each pregnant animal (usual litter size, three to four fetuses) were used for the determination of enzyme activities.

Each tissue sample was rinsed several times with ice-cold sucrose buffer, cut into small pieces, and then rinsed again with ice-cold buffer. A 20% (w/v) homogenate of liver or a 30% (w/v) homogenate of placenta was prepared in ice-cold sucrose buffer using a Potter–Elvehjem apparatus with a motor-driven Teflon® pestle. Each homogenate was filtered through gauze to remove adipose and connective tissue. Differential centrifugation of each homogenate was conducted at 4°, in which the nuclear, mitochondrial, microsomal and cytosolic fractions were isolated by centrifugation at 400 *g* for 10 min (nuclear pellet), 9,000 *g* for 15 min (mitochondrial pellet), and 105,000 *g* for 1 hr (microsomal pellet and cytosolic supernatant). Each pellet was resuspended in ice-cold sucrose buffer and filtered through gauze. The volumes of the four subcellular fractions were adjusted to be equivalent to that for a 10% (w/v) tissue homogenate (maternal liver and fetal liver) or a 30% (w/v) tissue homogenate (placenta). The samples were kept ice-cold and were analyzed within 36 hr.

Each subcellular fraction was incubated with sodium deoxycholate (four parts subcellular fraction and one part 10 mg/ml aqueous sodium deoxycholate) at 22° for 5 min to disrupt membranes. Aliquots (0.5 ml) of cytosolic fraction (ADH) and of each subcellular fraction (ALDH) were used to determine in duplicate the activity of ADH, low K_m ALDH and total ALDH by a modification of an established procedure [27], optimized for the guinea pig, in which NADH absorbance at 340 nm was measured at 22°. The activity of ADH was determined by measuring the oxidation of NADH with acetaldehyde as substrate. The reaction mixture (1.5-ml volume) contained 0.5 ml of cytosolic fraction, 50 mM KH_2PO_4 (pH 7.5), 0.15 mM NADH, 5 μM rotenone and 20 mM acetaldehyde. The activity of ALDH was determined by measuring the formation of NADH with acetaldehyde as substrate. The 1.5-ml reaction mixture contained 0.5 ml of subcellular fraction, 50 mM $Na_4P_2O_7$ (pH 7.4), 1.0 mM NAD^+, 0.10 mM pyrazole, 5 μM rotenone and either 25 μM acetaldehyde (low K_m ALDH activity) or 10 mM acetaldehyde (total ALDH activity). High K_m ALDH activity in each subcellular fraction was calculated as the difference between total and low K_m

ALDH activities for that fraction. Protein concentration in each subcellular fraction was determined by a protein-dye binding method [28] with bovine serum albumin used as the standard. ADH activity, determined in the cytosolic fraction only of each tissue sample, was expressed as nanomoles NADH oxidized per minute per gram of tissue. The low K_m ALDH and high K_m ALDH activities for each tissue sample, expressed as nanomoles NADH formed per minute per gram of tissue, were calculated as the sum of the respective enzyme activities in the four subcellular fractions. The protein concentration of each tissue sample, expressed as milligrams protein per gram of tissue, was calculated in the same way.

Disposition of ethanol and acetaldehyde in the maternal–fetal unit. Pregnant guinea pigs received a single oral dose of 0.5 g ethanol/kg maternal body weight at mean gestational age, 34 days (range, 33–35 days). The ethanol dose, as a 20% (v/v) solution in tap water, was intubated into the oral cavity by using a pediatric feeding tube over a 10-min period (experimental time, 0 min). Ethanol was administered between 9:00 and 11:00 a.m., and the animals had been fasted for 12 hr, with free access to water, before the ethanol dose to ensure optimal absorption. Animals (N = 4) were killed at each of the following experimental times: 15, 30, 45, 60, 90, 120 and 150 min. Maternal mixed arteriovenous blood was collected from the cervical stump. A sample of amniotic fluid was obtained when each fetus was delivered by cesarean section. Each fetus was rinsed with ice-cold saline solution, blotted dry, freeze-clamped with liquid-nitrogen-precooled tongs, and stored in liquid nitrogen. All samples were analyzed for ethanol and acetaldehyde on the day of experimentation by a headspace GLC procedure, in which artifactual acetaldehyde formation was minimized during sample preparation [29]. The lower limit of quantitative sensitivity of the method was 0.005 mg/ml (mg/g) for ethanol and 0.10 μg/ml (μg/g) for acetaldehyde. All samples were analyzed in duplicate.

Data analysis. Average values for the ethanol and acetaldehyde concentrations in fetal body and amniotic fluid of all the fetuses of each pregnant guinea pig were calculated. The ethanol and acetaldehyde concentration, enzyme activity and protein concentration data are presented as the mean ± SD. The data were analyzed by block-design analysis of variance or randomized-design, one-way analysis of variance followed by the Newman–Keuls test for a significant F statistic ($P < 0.05$), depending on which test was statistically appropriate. Two groups of data were considered to be significantly different when $P < 0.05$.

RESULTS

Optimal assay conditions for the measurement of ADH, low K_m ALDH and high K_m ALDH activities in the guinea pig were determined with respect to the substrate and cofactor concentrations, pH, and the use and concentrations of rotenone, an NADH oxidase inhibitor, and pyrazole, an ADH inhibitor (low K_m ALDH and total ALDH activities only). A

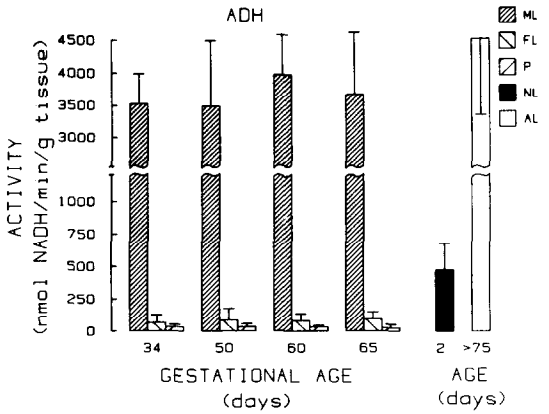

Fig. 1. Activity of alcohol dehydrogenase (ADH) in guinea pig maternal liver (ML), fetal liver (FL), placenta (P), neonatal liver (NL), and adult liver (AL). Data are presented as the mean ± SD (N = 6 at 34 days, N = 5 at 50 days, N = 6 at 60 days and N = 6 at 65 days of gestational age; N = 5 at 2 days and N = 8 at >75 days of age).

preliminary study of guinea pigs at 57–61 days of gestation demonstrated that ADH activity in maternal liver and fetal liver was 10–15 times greater when measured by the acetaldehyde reduction reaction (NADH oxidation) than by the ethanol oxidation reaction (NADH formation), and that enzyme activity in the placenta was measurable only with the acetaldehyde reduction reaction. Hence, ADH activity in the maternal–placental–fetal unit was measured only with the more sensitive acetaldehyde reduction reaction. This study also showed that ADH activity was localized in the cytosolic fraction; low K_m ALDH and high K_m ALDH activities were distributed among mitochondrial (preferentially), microsomal and cytosolic fractions for maternal liver and fetal liver, and between mitochondrial and microsomal fractions for placenta. The K_m of acetaldehyde for maternal hepatic cytosolic ADH was 3.5 mM. The K_m values of acetaldehyde for mitochondrial low K_m ALDH and mitochondrial high K_m ALDH were 0.30 μM and 0.66 mM, respectively, for maternal liver and 1.07 μM and 0.57 mM, respectively, for fetal liver.

The activities of ADH, low K_m ALDH and high K_m ALDH were determined in the maternal liver, fetal liver, and placenta of the pregnant guinea pig at 34, 50, 60 and 65 days of gestation, in the liver of the 2-day-old neonate, and in the liver of the adult male and non-pregnant female guinea pig (>75 days of age). The enzyme activity and protein concentration data were not significantly different between the adult male and non-pregnant female guinea pigs; the data were combined, and are reported as adult values. The ADH activities (Fig. 1) in adult liver and in maternal liver during gestation were similar. The fetal liver and placental ADH activities were low, did not change significantly during gestation, and were significantly less ($P < 0.05$) than adult hepatic enzyme activity. Neonatal liver enzyme activity was significantly less ($P < 0.05$) than adult hepatic enzyme activity, but was not significantly different from fetal liver enzyme activity

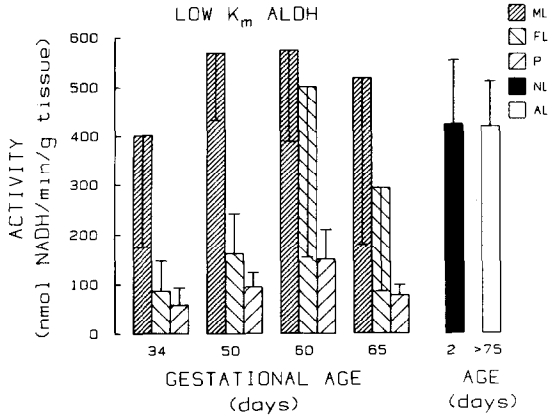

Fig. 2. Activity of low K_m aldehyde dehydrogenase (ALDH) in guinea pig maternal liver (ML), fetal liver (FL), placenta (P), neonatal liver (NL) and adult liver (AL). Data are presented as the mean ± SD (N values are given in the legend to Fig. 1).

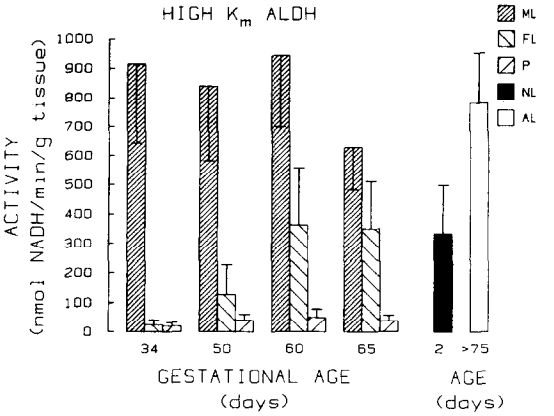

Fig. 3. Activity of high K_m aldehyde dehydrogenase (ALDH) in guinea pig maternal liver (ML), fetal liver (FL), placenta (P), neonatal liver (NL) and adult liver (AL). Data are presented as the mean ± SD (N values are given in the legend to Fig. 1).

at any gestational age. At each of the four gestational ages, maternal liver ADH activity was significantly greater ($P < 0.05$) than the enzyme activity in fetal liver and placenta; there was no significant difference between fetal liver and placental enzyme activities.

For low K_m ALDH activity (Fig. 2), there was no significant difference between adult liver and maternal liver. Fetal liver enzyme activity increased progressively throughout gestation; hepatic enzyme activity at 60 days of gestation was significantly greater ($P < 0.05$) than that at 34 and 50 days of gestation, but was not significantly different from the enzyme activity at 65 days of gestation, at 2 days of age, and at greater than 75 days of age. Placental enzyme activity was significantly less ($P < 0.05$) than adult hepatic enzyme activity and did not change significantly during gestation. At 34 and 50 days of gestation, low K_m ALDH activity was significantly greater ($P < 0.05$) in maternal liver than in fetal liver and placenta; fetal liver and placental enzyme activities were not significantly different. At 60 days of gestation, maternal liver and fetal liver enzyme activities were similar, but enzyme activity in maternal liver and fetal liver was significantly greater ($P < 0.05$) than in placenta. At 65 days of gestation, enzyme activity was significantly greater ($P < 0.05$) in maternal liver than in placenta, but was not significantly different between maternal liver and fetal liver.

For high K_m ALDH activity (Fig. 3), there was no significant difference between adult liver and maternal liver. Fetal hepatic enzyme activity at the four gestational ages and neonatal liver enzyme activity were significantly less ($P < 0.05$) than adult hepatic enzyme activity. However, hepatic enzyme activity at 34 and 50 days of gestation was significantly less ($P < 0.05$) than at 60 and 65 days of gestation and at 2 days of age. Placental enzyme activity was significantly less ($P < 0.05$) than adult hepatic enzyme activity, and did not change significantly during gestation. At each of the four gestational ages, high K_m ALDH activity was significantly

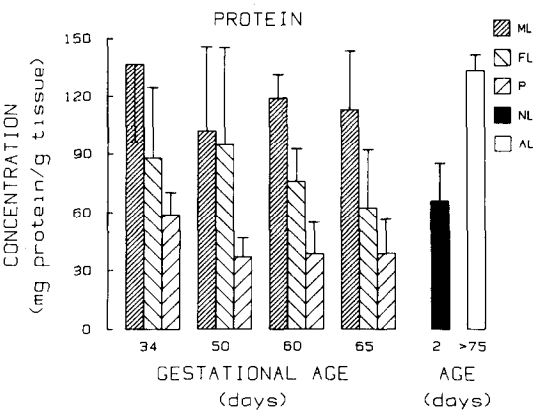

Fig. 4. Protein concentration in guinea pig maternal liver (ML), fetal liver (FL), placenta (P), neonatal liver (NL) and adult liver (AL). Data are presented as the mean ± SD (N values are given in the legend to Fig. 1).

greater ($P < 0.05$) in maternal liver compared with fetal liver and placenta. At 60 and 65 days of gestation, enzyme activity was significantly greater ($P < 0.05$) in fetal liver than in placenta.

The protein concentration (Fig. 4) of adult liver and maternal liver during gestation was not significantly different. Protein concentration in fetal and neonatal liver was significantly less ($P < 0.05$) than in adult liver. Protein concentration did not change significantly in fetal liver during gestation, but did decrease significantly ($P < 0.05$) in placenta after 34 days of gestation.

The ethanol and acetaldehyde concentration–time curves for maternal blood (MB), fetal body (FBY) and amniotic fluid (AF) of the pregnant guinea pig at mid-gestation (34 days of gestation) were determined after oral administration of 0.5 g ethanol/kg maternal body weight (Fig. 5). The MB ethanol concentration was maximal at 45 min and then decreased over the 60- to 150-min interval. The FBY

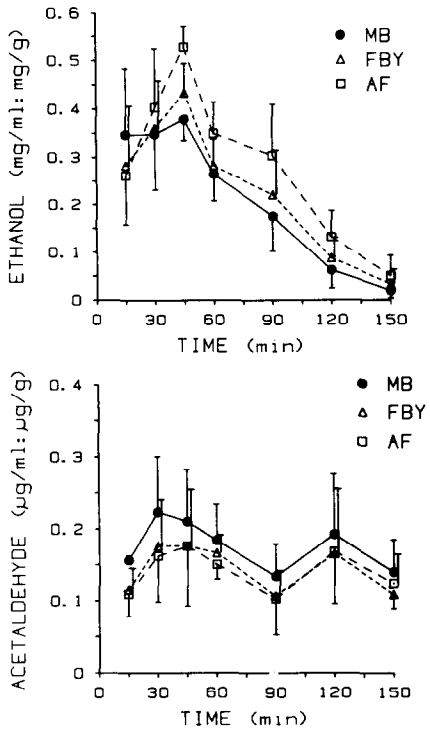

Fig. 5. Ethanol and acetaldehyde concentration–time curves in maternal blood (MB), fetal body (FBY) and amniotic fluid (AF) of the pregnant guinea pig at mid-gestation (34 days of gestation) after oral administration of 0.5 g ethanol/kg maternal body weight. Data are presented as the mean ± SD (N = 4 pregnant animals at each experimental time).

ethanol concentration was significantly lower ($P < 0.05$) than that in MB at 15 min, was maximal at 45 min, and then was similar to the MB ethanol concentration during the 60- to 150-min elimination phase. The AF ethanol concentration was significantly less ($P < 0.05$) than the MB ethanol concentration at 15 min, and then was significantly greater ($P < 0.05$) than the MB and FBY ethanol concentrations throughout the 30- to 150-min interval with maximal value at 45 min. The acetaldehyde concentrations in MB, FBY and AF were about 100- to 1000-fold less than the respective ethanol concentrations during the 150-min study. There were no consistent significant differences in acetaldehyde concentration among MB, FBY and AF.

The disposition of ethanol and acetaldehyde has been determined previously for oral administration of 0.5 g ethanol/kg maternal body weight to the near-term pregnant guinea pig (mean gestational age, 58 days; range, 56–59 days) [12]. The disposition of ethanol in MB, fetal blood and AF during near-term pregnancy was qualitatively similar to that at mid-gestation. The major difference involved the disposition of acetaldehyde, which was measurable only in MB during near-term pregnancy at a concentration that was about 1000-fold less than the MB ethanol concentration.

DISCUSSION

On the basis of the pharmacokinetics of ethanol in the maternal–fetal unit during near-term pregnancy [8–15], it has been hypothesized that the elimination of ethanol from the fetus is regulated primarily by maternal hepatic ADH-catalyzed biotransformation of ethanol. Our study in the guinea pig demonstrated that ADH activity was very low in fetal liver (1–2% of adult liver activity) and placenta (<1% of adult liver activity) throughout gestation. These findings are comparable to those for the rat, the only other mammalian species in which the ontogeny of ADH and ALDH has been determined [30]. Rat fetal liver ADH activity was only measurable (3% of adult liver activity) at 20 days of gestation (term, 21 days), and was not measurable in placenta. In view of these data and the pharmacokinetics of ethanol in the maternal–fetal unit at mid-gestation and near-term pregnancy, characterized by similar maternal and fetal ethanol concentration–time curves, it would appear that the elimination of ethanol from the conceptus at all gestational ages is regulated primarily by maternal hepatic ADH-catalyzed biotransformation of ethanol.

In the 2-day-old neonatal guinea pig, hepatic ADH activity was about 10% of adult liver activity, which is similar to hepatic ADH activity in the 2-day-old neonatal rat (about 20% of adult liver activity) [30], and hepatic ADH activity in the 9-day-old human neonate (about 15% of adult liver activity) [31]. These data indicate that, in the neonate, ADH-catalyzed biotransformation of ethanol would be slow, thereby resulting in slow elimination of ethanol from the systemic circulation, as has been reported for the human neonate [25], neonatal rhesus monkey [9], and neonatal lamb [21].

Data on the pharmacokinetics of ethanol-derived acetaldehyde during near-term pregnancy [11–15] led to the hypothesis that there is preferential development of fetal liver low K_m ALDH activity, which oxidizes acetaldehyde transferred from the maternal blood circulation. In the guinea pig, fetal hepatic low K_m ALDH activity increased progressively during gestation, such that fetal hepatic enzyme activity at 34 days of gestation was 21% of adult liver activity and enzyme activity at 60 days of gestation was similar to adult activity. Placental low K_m ALDH activity, however, was low (14–36% of adult hepatic activity). For ethanol administration to the pregnant guinea pig at mid-gestation, the maternal and fetal acetaldehyde concentrations were similar. In contrast, for ethanol administration to the near-term pregnant guinea pig, acetaldehyde was present in maternal blood, but was not measurable in fetal blood [12]. Thus, there appears to be an inverse relationship between the development of low K_m ALDH activity in fetal liver and the concentration of acetaldehyde in the fetus relative to the mother. Furthermore, these data demonstrate that there is preferential development of hepatic low K_m ALDH activity relative to ADH activity, which would protect the conceptus from exposure to ethanol-derived acetaldehyde.

In the rat, fetal hepatic low K_m ALDH activity at 15 and 20 days of gestation was 5 and 16%, respectively, of adult hepatic activity, and placental enzyme activity at 15 and 20 days of gestation was 7

and 2%, respectively, of adult hepatic activity [30]. In the 2-day-old neonatal guinea pig, hepatic low K_m ALDH activity was similar to adult liver activity, whereas in the 2-day-old neonatal rat, hepatic low K_m ALDH activity was about 40% of adult liver activity [30]. Hence, there would appear to be more prenatal development of hepatic low K_m ALDH activity in the guinea pig compared with the rat, which may be related to the longer period of gestation for the guinea pig.

In the guinea pig, fetal liver high K_m ALDH activity increased to approximately 45% of adult hepatic enzyme activity at 65 days of gestation, whereas placental high K_m ALDH activity was low (3–6% of adult hepatic activity). The 2-day-old neonatal liver high K_m ALDH activity was the same as that in the fetal liver at late gestation. In the rat, fetal hepatic high K_m ALDH activity was 4 and 15% of adult activity at 15 and 20 days of gestation, respectively, and placental enzyme activity at 15 and 20 days of gestation was 7 and 4%, respectively, of adult hepatic enzyme activity [30]. There was, however, a pronounced increase in rat hepatic high K_m ALDH activity after parturition, with activity at 2 days of age that was 50% of adult hepatic enzyme activity. Hence, there would appear to be more prenatal development of hepatic high K_m ALDH activity in the guinea pig compared with the rat, as apparently is the case for low K_m ALDH activity. It is important to note that high K_m ALDH would only be involved in the oxidation of ethanol-derived acetaldehyde if there was a genetic deficiency or drug-induced inhibition of low K_m ALDH. Under these conditions, there would be increased acetaldehyde concentration in the mother and fetus, in view of the markedly different K_m values for acetaldehyde of the low K_m ALDH (μM acetaldehyde) and high K_m ALDH (mM acetaldehyde) isozymes.

In conclusion, the ontogeny of the activities of ADH and ALDH in the liver and placenta of the guinea pig was elucidated in this study. The data indicate that: (a) the fetus has virtually no capacity to oxidize ethanol, and is virtually unprotected from the direct toxic effects of ethanol; (b) during gestation, the fetus has increasing capacity to oxidize ethanol-derived acetaldehyde, and would appear to be increasingly protected from acetaldehyde-induced toxicity as pregnancy progresses; and (c) the neonate has little capacity to oxidize and eliminate ethanol, but does have appreciable capacity to oxidize ethanol-derived acetaldehyde, which could result in prolonged exposure to ethanol and its toxic effects.

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REFERENCES

1. Abel EL (Ed.), *Fetal Alcohol Syndrome, Vol. II, Human Studies*. CRC Press, Boca Raton, FL 1982.
2. Abel EL (Ed.), *Fetal Alcohol Syndrome, Vol. III, Animal Studies*. CRC Press, Boca Raton, FL 1982.
3. McLeod W, Brien J, Loomis C, Carmichael L, Probert C and Patrick J, Effect of maternal ethanol ingestion on fetal breathing movements, gross body movements, and heart rate at 37 to 40 weeks' gestational age. *Am J Obstet Gynecol* **145**: 251–257, 1983.
4. Patrick J, Richardson B, Hasen G, Clarke D, Wlodek M, Bousquet J and Brien J, Effects of maternal ethanol infusion on fetal cardiovascular and brain activity in lambs. *Am J Obstet Gynecol* **151**: 859–867, 1985.
5. Richardson BS, Patrick JE, Bousquet J, Homan J and Brien JF, Cerebral metabolism in fetal lamb after maternal infusion of ethanol. *Am J Physiol* **249**: R505–R509, 1985.
6. Webster WS, Walsh DA, McEwen SE and Lipson AH, Some teratogenic properties of ethanol and acetaldehyde in C57BL/6J mice. Implications for the study of the fetal alcohol syndrome. *Teratology* **27**: 231–243, 1983.
7. Pierce DR and West JR, Blood alcohol concentration: a critical factor for producing fetal alcohol effects. *Alcohol* **3**: 269–272, 1986.
8. Ng PK, Cottle MK, Baker JM, Johnson B, van Muyden P and van Petten GR, Ethanol kinetics during pregnancy. Study in ewes and their fetuses. *Prog Neuropsychopharmacol Biol Psychiatry* **6**: 37–42, 1982.
9. Hill DE, Slikker W Jr, Goad PT, Bailey JR, Sziszak TJ and Hendrickx AG, Maternal, fetal, and neonatal elimination of ethanol in nonhuman primates. *Dev Pharmacol Ther* **6**: 259–268, 1983.
10. Cumming ME, Ong BY, Wade JG and Sitar DS, Maternal and fetal ethanol pharmacokinetics and cardiovascular responses in near-term pregnant sheep. *Can J Physiol Pharmacol* **62**: 1435–1439, 1984.
11. Brien JF, Clarke DW, Richardson B and Patrick J, Disposition of ethanol in maternal blood, fetal blood, and amniotic fluid of third-trimester pregnant ewes. *Am J Obstet Gynecol* **152**: 583–590, 1985.
12. Clarke DW, Steenaart NAE and Brien JF, Disposition of ethanol and activity of hepatic and placental alcohol dehydrogenase and aldehyde dehydrogenases in the third-trimester pregnant guinea pig for single and short-term oral ethanol administration. *Alcoholism: Clin Exp Res* **10**: 330–336, 1986.
13. Clarke DW, Steenaart NAE, Slack CJ and Brien JF, Pharmacokinetics of ethanol and its metabolite, acetaldehyde, and fetolethality in the third-trimester pregnant guinea pig for oral administration of acute, multiple-dose ethanol. *Can J Physiol Pharmacol* **64**: 1060–1067, 1986.
14. Brien JF, Clarke DW, Smith GN, Richardson B and Patrick J, Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe. *Am J Obstet Gynecol* **157**: 204–211, 1987.
15. Clarke DW, Smith GN, Patrick J, Richardson B and Brien JF, Disposition of ethanol and its proximate metabolite, acetaldehyde, in the near-term pregnant ewe for short term maternal administration of moderate-dose ethanol. *Drug Metab Dispos* **16**: 464–468, 1988.
16. Brien JF and Loomis CW, Pharmacology of acetaldehyde. *Can J Physiol Pharmacol* **61**: 1–22, 1983.
17. O'Shea KS and Kaufman MH, The teratogenic effect of acetaldehyde: implications for the study of the fetal alcohol syndrome. *J Anat* **128**: 65–76, 1979.
18. O'Shea KS and Kaufman MH, Effect of acetaldehyde on the neuroepithelium of early mouse embryos. *J Anat* **132**: 107–118, 1981.
19. Blakley PM and Scott WJ Jr, Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 1. Teratogenicity of ethanol and acetaldehyde.

Toxicol Appl Pharmacol **72**: 355–363, 1984.
20. Khanna JM and Israel Y, Ethanol metabolism. *Int Rev Physiol* **21**: 275–315, 1980.
21. Cumming ME, Ong BY, Wade JG and Sitar DS, Ethanol disposition in newborn lambs and comparison of alcohol dehydrogenase activity in placenta and maternal sheep, fetal and neonatal lamb liver. *Dev Pharmacol Ther* **8**: 338–345, 1985.
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. Nishimura H and Shiota K, Summary of comparative embryology and teratology. In: *Handbook of Teratology* (Eds. Wilson JG and Fraser FC), Vol. 3, pp. 119–154. Plenum Press, New York, 1977.
24. Sisk DB, Physiology. In: *The Biology of the Guinea Pig* (Eds. Wagner JE and Manning PJ), pp. 63–98. Academic Press, New York, 1976.
25. Idanpaan-Heikkila J, Jouppila P, Akerblom HK, Isoaho R, Kauppila E and Koivisto M, Elimination and metabolic effects of ethanol in mother, fetus, and newborn infant. *Am J Obstet Gynecol* **112**: 387–393, 1972.
26. Draper RL, The prenatal growth of the guinea-pig. *Anat Rec* **18**: 369–392, 1920.
27. Tottmar SOC, Pettersson H and Kiessling K-H, The subcellular distribution and properties of aldehyde dehydrogenases in rat liver. *Biochem J* **135**: 577–586, 1973.
28. Bradford MM, 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.
29. Steenaart NAE, Clarke DW and Brien JF, Gas-liquid chromatographic analysis of ethanol and acetaldehyde in blood with minimal artifactual acetaldehyde formation. *J Pharmacol Methods* **14**: 199–212, 1985.
30. Sjoblom M, Pilstrom 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.
31. Pikkarainen PH and Raiha NCR, Development of alcohol dehydrogenase activity in the human liver. *Pediat Res* **1**: 165–168, 1967.