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ETHANOL, OXIDATIVE STRESS, REACTIVE ALDEHYDES, AND THE FETUS

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1. ABSTRACT

The fetotoxic effects of maternal ethanol (E) consumption have been documented for over two decades, yet the mechanisms underlying this devastating phenomenon remain uncertain. The wide variety of cellular/biochemical effects of E on fetal tissues is itself a puzzle and strongly suggests that fetotoxic responses to E reflect a multifactorial setting. Many of these responses can be conceptually connected to effects on membrane structure and function. Representative of this, are studies in our laboratory documenting E effects on fetal cell replication, membrane transport systems, membrane fluidity, Na^+-K^+ pump expression, and EGF receptor expression. Recent studies have provided evidence that oxidative stress may be one mechanism by which E produces these membrane-related events. We initially observed E-induced oxidative stress in cultured fetal rat hepatocytes, the latter exhibiting morphological and biochemical signs of mitochondrial damage. E increased H_2O_2, O_2^-, lipid peroxidation products, along with signs of membrane damage. Supplementation with antioxidants or agents that enhance glutathione stores reversed these effects. E was found to inhibit activities of mitochondrial respiratory chain components (a potential source of the enhanced levels of H_2O_2, and O_2^-) and this could be reversed by antioxidant treatment. Subsequent studies have documented oxidative stress and membrane lipid peroxidation in fetal brain and liver (gestation day 19) following a two day maternal E consumption and in gestation day 14 and 17 "embryos" immediately following a single dose of E to the pregnant dam.

The means by which E can induce oxidative stress in fetal cells is under investigation. We have examined effects of E on activities of key antioxidant enzymes and found no depressant responses. However, the low levels of antioxidants in fetal tissues and an exaggerated response of fetal mitochondria to prooxidant stimulation *in vitro*, suggest that fetal cells are strongly predisposed to oxidative stress. Additionally, recent studies have suggested that fetal tissues are likewise prone to the formation and subsequent accumulation of at least one toxic lipid peroxidation product, 4-hydroxynonenal.

We conclude that maternal E consumption induces oxidative stress in fetal tissues and that this is responsible for some toxic responses to E. Additionally, the low antioxidant defenses in fetal tissues and accumulation of toxic aldehyde products of lipid peroxidation predispose the fetus to oxidative damage.

2. INTRODUCTION

A major cause of birth defects today is maternal consumption of ethanol. The maximal expression of this teratogenic response is the Fetal Alcohol Syndrome (FAS), first labeled as such in 1972 (1) and first, formally reported by Lemoine, P., *et al* (2). These and subsequent accounts (3) reported high incidence of pre- and postnatal growth retardation, developmental delay, a specific pattern of craniofacial abnormalities and limb and cardiac defects. Currently, diagnosis of this syndrome is characterized by 1.

growth retardation, 2. CNS abnormalities (which may include abnormal brain morphology, neurological abnormalities, developmental and intellectual impairment), and 3. the characteristic pattern of craniofacial abnormalities (1-3). These are mostly associated with sustained heavy maternal ethanol consumption. However, a number of epidemiological studies over the past decade have provided evidence that more moderate maternal ethanol consumption may be connected with lasting behavioral and intellectual dysfunctions (3). The minimal amount of consumed ethanol needed to elicit adverse outcomes is and will likely continue to be controversial, due to the complex nature of consumption patterns, difficulties in documenting use, and the plethora of other social and concomitant drug use variables. Also, the pattern of consumption may be relevant. A 1990 prospective study (4) found "binge" consumption of five or more drinks on at least one occasion to be associated with lasting effects on IQ and learning problems.

Our initial efforts in this field utilized the Sprague Dawley rat either treated with ethanol (via gastric intubation) at specific times during gestation to mimic a "binge" setting or fed the Lieber DeCarli liquid diet throughout gestation as a "chronic" model (5,6). A consistent finding in both the ethanol-exposed rodent fetus and neonate was decreased body weight combined with parallel decreases in heart, liver, and kidney growth. Brain weight was also decreased if the fetus had been exposed to ethanol throughout gestation or to a 3 day binge late in gestation. These studies were in the middle to late 1970s and this period marked the emergence of a large number of reports detailing (describing) the impact of ethanol on fetal and neonatal growth and development. Most models utilized rodents with an ethanol-related growth inhibition being reported for both mice and rats (6,7) by far more laboratories than can be cited here. Maternal ethanol intake by nonhuman primates has also been reported to generate reduced fetal growth but no abnormal facial characteristics were detected (8).

3. MECHANISMS UNDERLYING FETAL ALCOHOL SYNDROME

The most striking aspect of a review of the FAS literature is the extensive variety of effects that ethanol has on fetal tissue (9). How a single compound such as ethanol could directly effect such a diverse range of cellular/biochemical events is puzzling and seems unlikely. It makes far more intuitive sense if ethanol were to primarily alter one or two basic cellular processes (eg membrane integrity, energy production and/or produce factors which secondarily affect these basic processes) that are key to cellular order. Such a "disruption" would then produce the observed secondary cascade of events often credited to the drug itself. A central causal factor has not been confirmed, but the fetus occupies a unique setting in which its viability is totally dependent upon its host (the mother), many of whose biological systems are also affected by ethanol. Thus, when probing the mechanistic basis for FAS, we are likely addressing a multifactorial or, at least dual, event in which the fetotoxic effects of ethanol may be derived from direct effects both on fetal cells (these key cellular processes) as well as via secondary effects on the maternal/placental functions.

3.1. Indirect Effects: Fetal hypoxia

This school of thought is derived from studies indicating that maternal ethanol intake may restrict placental blood supply, thereby reducing oxygen supply to the fetus (10,11). Infusing ethanol (2 to 4 g/kg) into the monkey has been found to reduce fetal blood pressure, heart rate, and induce acidosis (12). In the rat, microsphere studies confirm an ethanol-related decrease in placental blood flow (13). This concept has not been confirmed by the one Doppler study done in humans, however the dose and duration of exposure to ethanol were well below those thought to be overtly teratogenic and the patient did not serve as her own control (14). A role for Prostaglandins. Prostanoids are established mediators of pregnancy and are requisite for "normal" fetal development (15). However, exogenously administered prostaglandin (PGE) can be teratogenic (16) and compounds which inhibit PGE synthesis have been shown to offer protective effects against the fetotoxic effects of ethanol (17-19). Accordingly, prostanoids may play a causal role in FAS. This could be a direct effect on fetal tissues or it may be connected to altered regulation of umbilical and placental blood flow. PGE is produced by both the umbilical and placental vessels whose blood flow is regulated by prostacyclin/thromboxane actions (20,21) and PGE inhibitors reduce oxygen-induced constriction of umbilical arteries (22). The relevance of this to the current discussion is the possibility of reperfusion-related injury and associated oxidative stress that could be generated in the maternal-fetal axis by disruptions of utero/placental blood flow.

3.2. Direct Effects on Fetal Tissue

Ethanol exposure in the absence of maternal influences produces a variety of toxic and potentially toxic responses in fetal tissues. A fully comprehensive discussion of these is not germane to the present review (see another review for many of these (9), however we will present several previously documented adverse responses to ethanol that might be derived from ethanol-mediated oxidative stress. Potentially important, may be that many of the effects of ethanol on cultured fetal cells that we have observed can be connected to alterations in membrane structure and/or function.

Cell replicative capacity and EGF receptor dynamics. Cell replicative capacity is clearly a central factor in fetal growth and development. Cell replication is, in turn, subject to autocrine, paracrine and endocrine control by a variety of growth factors. Of the many peptide growth factors regulating cell replication, epidermal growth factor (EGF) has been implicated in growth regulation at virtually all stages of development from the blastocyst (23) to the neonate (24). In our hands, the rat fetal hepatocyte cultured in defined media requires only EGF (optimally 2 ng/ml) to replicate and ethanol blocks this replication (25). This replicative block is accompanied by increased surface expression of both high and low affinity EGF receptors

which may be caused by impaired internalization of the occupied EGF receptor (26). This is a response also produced in cultured fetal rat hepatocytes by another compound that blocks replication, TGF_{Beta1} (27).

Membrane fluidity and composition. There is an extensive body of evidence that ethanol alters fluidity and composition of various cell membranes (28). This has been linked, albeit not conclusively, to altered conformation of membrane proteins, which could include such key elements as growth factor receptors, enzymes, and neurohormone receptor complexes (29). While ethanol-related alterations in cell membrane fluidity occur, their functional significance remains to be established. However, membrane fluidity changes as factors in the pathogenesis of FAS are intuitively attractive in that they could represent a single underlying mechanism by which ethanol may generate such a wide variety of altered membrane events requisite for cell viability and function. In this regard, we have found that in cultured fetal hepatocytes exposed to ethanol, measures of membrane fluidity are altered in parallel with changes in membrane-related functions such as Na^+-K^+ pump expression (30) and EGF receptor internalization (26).

4. OXIDATIVE STRESS, LIPID PEROXIDATION, AND REACTIVE ALDEHYDE PRODUCTION

Oxygen, while central to life, is potentially toxic. In its ground state, it possesses two unpaired electrons with parallel electronic spins. Such a setting makes a two electron reduction kinetically unlikely, however sequential one-electron reductions do occur, generating oxygen free radicals. In the biological setting, the initial one electron exchange generates the superoxide anion radical (O_2^-). The protonated two electron reduction produces H_2O_2 (the protonated form of the peroxide ion) with the final protonated four electron product being water. Oxygen free radicals are known to adversely affect a variety of cellular elements, proteins, DNA bases and sugars, polysaccharides and lipids. Since much of our previous work has documented ethanol effects on fetal cell membranes (25,26,30-32) and microscopic examinations of ethanol exposed fetal cells indicated extensive mitochondrial membrane damage, the issue of ethanol-related oxidative damage to fetal cell membranes (eg lipid peroxidation) became a major interest. It is well documented that oxygen free radicals react with unsaturated membrane lipids, initiating a self-perpetuating peroxidation process (33). This reaction can produce loss of membrane function and ultimately cell death if the damage is sufficient. Ethanol has been shown to induce lipid peroxidation (34,35) and its effect on mitochondrial morphology (inner membrane damage) in our fetal hepatocytes originally led us to suspect that ethanol produced oxidative stress in these cells. The studies outlined in the following sections confirm this.

4.1. Ethanol and Oxidative Stress in the Fetus

4.1.1. Oxidative stress and the fetus

There has previously been little experimental evidence linking maternal ethanol consumption to oxidative damage of the fetus. To our knowledge, this resides in two reports by Dreosti, et al.(36,37) in which dams were provided a 20% ethanol solution as a sole source of water throughout gestation. This regimen increased malondialdehyde (MDA) content of fetal liver mitochondria (as we have observed in cultured fetal hepatocytes (10)) but had no effect on MDA content of the microsomal fraction of fetal liver or on either fetal or maternal brain. The 2 day "binge" regimen that we have utilized estimated MDA in post nuclear fractions rather than the two organelle enriched isolates (38), but it is in general agreement with the prior report (36) except that we found both MDA and dienes to be increased in fetal brain. Evidence that might indirectly support ethanol-induced oxidative stress in the fetus was included in a report by Reyes, E., *et al* (39), which indicated that chronic maternal ethanol consumption can reduce GSH content of fetal brain and liver. Our short term exposure regimen (outlined below) supported this, but the earlier report by Dreosti, *et al* (37) found no evidence that chronic consumption of ethanol altered GSH of either fetal liver or brain. Once again, varied ethanol intake patterns used by these two groups (ethanol in drinking water vs ethanol in a liquid diet) might explain this difference.

4.1.2. Ethanol-induced oxidative stress in cultured fetal hepatocytes

Our prior studies with cultured FRH illustrated that ethanol-related increases in H_2O_2, O_2^- and membrane lipid peroxidation were paralleled by signs of cell membrane damage (leakage of LDH, ALT, ^{54}Cr) and blockade of cell replication (25,38). The enhanced cellular leakage implies damage to cell membrane components that could compromise fetal cell function. This was accompanied by increased levels of two markers of lipid peroxidation, MDA and conjugated dienes (figure 1). Additionally, the morphological and biochemical signs of mitochondrial damage were associated with depressions of complex I and IV activities along with decreased ATP synthesis (40). A conceivable importance of the latter ethanol effect is that inhibition of mitochondrial respiratory chain components has been shown to stimulate production of reactive species, hence this could be the origin of the increased levels of H_2O_2 and O_2^- (38,41). It is relevant that this occurs in a tissue with microsomal P450 systems that are so poorly developed that they likely contribute little to the generation of reactive oxygen species. Potentially of greater significance is the inhibitory effect of ethanol on cultured fetal cell replication/growth as this could be the mechanism underlying the numerous observations of impaired growth and replication of fetal and neonatal brain cells associated with *in utero* ethanol exposure (42-44). Importantly, the ethanol induced block of cell replication and concomitant membrane lipid peroxidation could both be mitigated by augmenting cellular antioxidant capacity (figure 1) along with reversal of the impaired mitochondrial respiratory chain components when mitochondrial reduced glutathione (GSH) levels were normalized (38). This is strong documentation that ethanol induced oxidative stress can impact negatively on vital fetal cell functions (replication, membrane integrity, and mitochondrial energy production) and it implies that compromised antioxidant status could be one underlying mechanism.

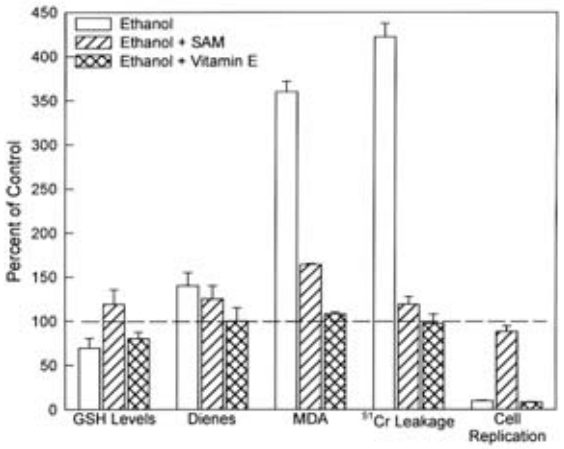

Figure 1. Ethanol effects, oxidant stress, and antioxidant supplementation on cultured fetal rat hepatocytes. Cells were cultured with or without ethanol (2 mg/ml) for 24 hours. Antioxidants included with some samples were s-adenosylmethionine (0.1 mMolar) to normalize cellular reduced glutathione (GSH) and vitamin E (alpha-tocopherol) (0.1 mMolar) (ref. 38). Values are expressed as means +/- SEM for 5 to 7 values per point. These data were presented, in part in Hepatology 18, 648-659, 1993; Permission granted.

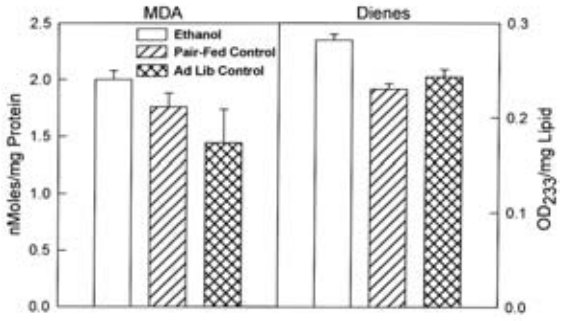

Figure 2. Brain MDA formation following a 2 day in utero ethanol exposure. Dams (Sprague Dawley rats) were intubated with ethanol (4 gm/kg) at 12 hour intervals on days 17 and 18 of gestation and one hour prior to sacrifice on gestation day 19. Pair-fed controls received isocaloric dextrose to balance the ethanol and lab chow according to the ethanol treated rat to which they were paired. Ad lib control rats received lab chow. n= 12 pregnancies; 3 assays per pregnancy (ref.55). Values are expressed as means +/- SEM. These data were presented, in part in Alcoholism: Clinical and Experimental Research 19, 714-720, 1995; Permission granted.

4.1.3. Ethanol-induced oxidative stress in the fetus

The ultimate effects of an ethanol-induced prooxidant setting and the resulting enhanced membrane lipid peroxidation on fetal development and viability has yet to be established. However, the potential for damage is substantial. It is well documented that the fetus/embryo is exquisitely sensitive to oxidative stress, the generation of which can cause a spectrum of responses ranging from structural malformations to embryonic death (45-47). Some or much of this might be related to lipid peroxidation since its products (such as reactive aldehydes) and the intermediate radicals formed in the peroxidation process are known to adversely affect a variety of cellular functions key to growth. These later events, which are also observed with ethanol or acetaldehyde exposure, include cytoskeletal disruption (48,49), mitochondrial dysfunction (50-53), and alteration of membrane protein receptors and subsequent signal transduction (31,54).

Recent studies in our laboratory have documented evidence of oxidant stress in fetal brain and liver following short-term maternal ethanol consumption (55). Pregnant rats were administered ethanol (4 gm/kg) by gavage twice a day on days 17 and 18 of gestation and sacrificed on day 19 one hour following a final dose of ethanol. This "binge" model increased both MDA and conjugated diene levels in fetal brain (figure 2) and in fetal liver. This treatment modestly decreased GSH in both fetal brain and liver (19% and 10%, respectively) but had no effect on vitamin E content of either organ. Additionally, it was found that a single exposure to ethanol, in utero, was sufficient to increase MDA levels in whole "embryos" at earlier stages of gestation, days 17 and 14 (figure 3 for day 14). Thus, short-term in utero ethanol exposure can elicit oxidative stress in the fetus, however the origin of this and its effects on fetal development remain to be established. It is difficult, at this juncture, to connect the small decreases in brain and liver GSH following this ethanol exposure regimen to the observed oxidative stress. However, long-term *in utero* ethanol exposure might generate a greater GSH depletion that could generate or exacerbate oxidant stress in the fetus.

4.2.Antioxidant Defenses and the Fetus

4.2.1. An absence of reduced antioxidant defenses following short-term ethanol exposure

There is substantial evidence that ethanol can deplete at least some important antioxidant defense systems. With respect to hydrophilic nonenzymatic antioxidants, there are reports that ascorbic acid excretion is stimulated by ethanol (56) and that ethanol decreases tissue GSH (57,58). Additionally, ethanol consumption (acute and chronic) has been reported to decrease alpha -tocopherol content of rat liver (59) and human plasma (60). Clearly, these could be factors contributing to our observations of oxidative damage to membrane lipids following maternal ethanol intake, however no clear cause and effect connections between these events have been established. We have not yet monitored ascorbate in fetal tissues, however our ethanol exposure regimen did not decrease alpha -tocopherol levels in fetal brain or liver and the effect of the modest 10% to 19% depletion of total organ GSH cellular antioxidant defenses is probably slight, at best. More recent studies have investigated effects of the two day ethanol exposure regimen on activities of fetal liver or brain catalase, superoxide dismutase (both cytosolic and mitochondrial), glutathione peroxidase, and glutathione S-transferase (61). No inhibitory effects were found. To the contrary, activities of these antioxidant enzymes were mostly enhanced, a setting often seen in oxidative stress.

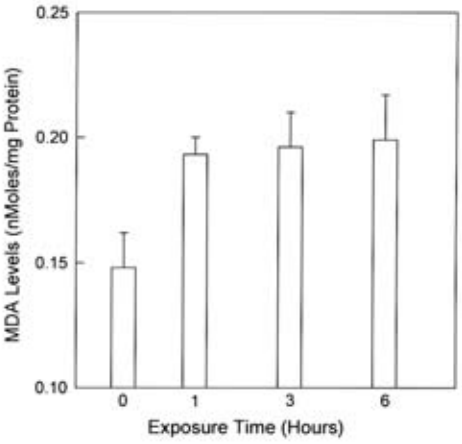

Figure 3. Effect of a single in utero exposure to ethanol on MDA content of day 14 whole embryos. Dams (Sprague Dawley rats) were intubated with ethanol (4 gm/kg) and sacrificed at 2, 3, or 6 hours thereafter. Malondialdehyde (MDA) levels were assayed in homogenates of whole embryos grouped for each pregnancy. n= 20 dams and 228 embryos; 5 dams per point; 3 assay values per pregnancy (ref. 55). Values are expressed as means +/- SEM. These data were presented, in part in Alcoholism: Clinical and Experimental Research 19, 714-720, 1995; Permission granted.

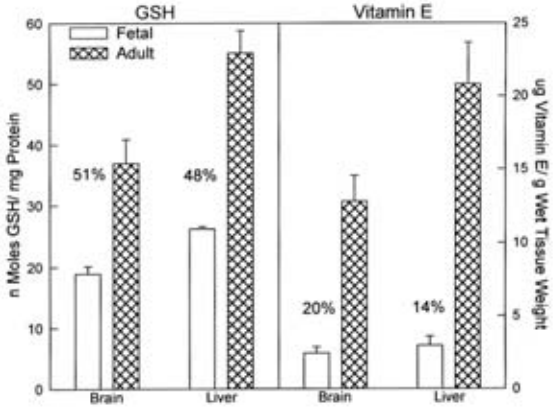

Figure 4. Comparison of GSH and vitamin E content of fetal and adult brain and liver. Dams (Sprague Dawley rats) were sacrificed on gestation day 19. GSH and vitamin E were assayed in homogenates of brain and liver. n= 9 sets of tissues and pregnancies for GSH measures and 8 for vitamin E (ref. 55). Values are expressed as means +/- SEM. These data were presented, in part in Alcoholism: Clinical and Experimental Research 19, 714-720, 1995; Permission granted.

4.2.2. Developmentally compromised antioxidant status predisposes the fetus to oxidative stress

Fetal tissues generally possess lower activities and levels of oxidative defenses than the adult (62,63) and might thereby be more sensitive to oxidative stress produced by ethanol. The former was confirmed by our studies. alpha-tocopherol levels in 19 day fetal brain and livers are only 14% to 20% of those in the corresponding adult tissues (figure 4) while GSH content of these two fetal organs were about one half of adult values (figure 4) (61). Similar patterns exist for most of the key antioxidant enzyme systems in brain and liver. In the day 19 fetal brain, glutathione S-transferase and glutathione peroxidase activities are only 41% and 12% of adult values, respectively. In the fetal liver, this pattern is respectively, 31% and 11% of activities in adult liver (figure 5) (61). Clearly, such low antioxidant defenses could predispose these fetal tissues to oxidative stress and the resulting damage.

4.3. 4-Hydroxynonenal, its toxic effects and its generation by ethanol

4.3.1. Oxidative Stress, lipid peroxidation, and 4-hydroxynonenal production

Aldehydes are generated as by-products of lipid peroxidation, a complex, self sustaining reaction in which polyunsaturated fatty acids (PUFAs) of the membrane bilayer are converted to reactive/unstable lipid hydroperoxides (figure 6) (64). Secondarily, these compounds can be converted to a wide variety of products (such as aldehydo-, keto-,epoxy-) by scission, fission, rearrangement, and oxidative reactions (64). There are a variety of aldehydes produced by chain cleavage and recurrent oxidative reactions (64,65), with one of the best studied groups being 4-hydroxyalkenals, especially 4-hydroxynonenal (HNE). The greatest yields of HNE have been shown to be generated from linoleate and arachidonic acids (figure 6), thus one might expect membrane fractions rich in these two PUFAs to be the most facile in producing HNE. This could be relevant to the ethanol exposed fetus as short-term ethanol exposure has been reported to increase PUFA content of adult liver mitochondria (66) and there are a variety of gestational changes in fetal tissue phospholipid molecular species (67). Additionally, HNE is metabolized by enzyme species that are less well developed in the fetus than in the adult (see below).

4.3.2. Toxic responses to HNE.

HNE is generally considered to be the most toxic aldehydic product of lipid peroxidation (for a review see Esterbauer, H., *et al* (68)) due to its high reactivity towards cellular macromolecules (69-71). Thus, there is documentation of a wide variety of toxic "functional" responses to HNE. Most germane to the fetal setting are effects that can be connected to cell replication/cell cycle progression and mitochondrial function. HNE produces replicative block of cultured neuroglial cells, possibly caused by crosslinking to cytoskeletal proteins (72), alters cell cycle progression and granulocytic differentiation (73), modifies monocyte migration and chemotactic responses (74), and inhibits synthesis of DNA, RNA and protein (75,76). Studies by a co-investigator on this proposal and others have documented a variety of effects of HNE on key mitochondrial functions. These include inactivation of enzymes such as Na^+-K^+-ATPase and adenine nucleotide translocator (77,78), inhibition of mitochondrial transcription machinery (79) and alteration of mitochondrial membrane fluidity (80). With respect to ethanol, there is evidence from both clinical observation and laboratory studies that exposure to ethanol causes tissue damage and dysfunction, particularly in the liver,

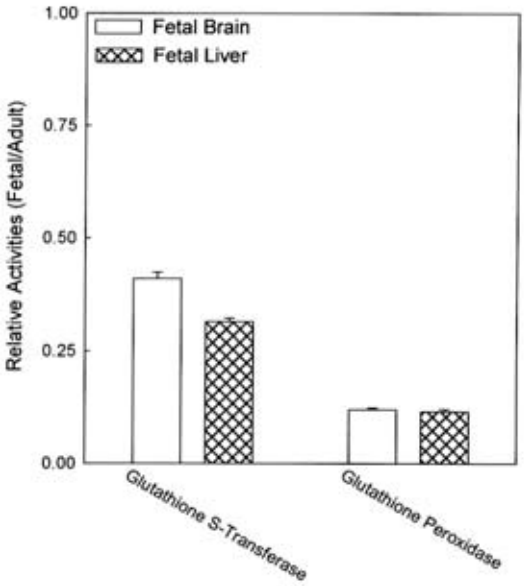

Figure 5. Comparison of glutathione-dependent antioxidant enzyme activities in fetal and adult brain and liver. Dams (Sprague Dawley rats) were sacrificed on gestation day 19. Enzyme activities were determined in homogenates of brain and liver. n= 10 values and assays from tissues obtained from 10 pregnancies (ref. 61 for the liver data). Values are expressed and mean +/- SEM. These data were presented, in part in Alcohol 13:1-6, 1996; Permission granted.

Figure 6. Formation of 4-hydroxynonenal from linoleic and arachidonic acid components of cell lipids.

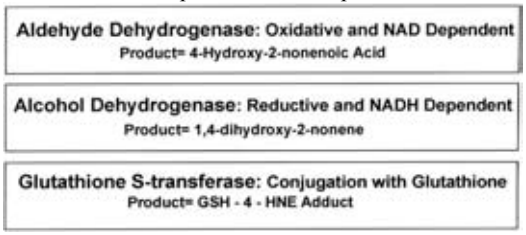

Figure 7. Metabolism of 4-hydroxynonenal in liver by three enzyme systems.

with the mitochondria being target organelles (81-83). The mechanisms underlying these hepatotoxic effects of ethanol remain unclear, however there is tantalizing evidence that oxidative stress and subsequent generation of aldehyde peroxidation products produced (HNE specifically), play a role (84-87). Studies in our laboratory have found that *in utero* and *ex utero* ethanol exposure increases HNE levels in fetal and neonatal liver.

4.3.3. Production of HNE in fetal liver exposed to ethanol

Recent studies in our laboratory have found that a two day "Binge" exposure to ethanol strikingly enhances HNE content of whole fetal liver and of liver mitochondria. This exposure regimen increased HNE content of fetal liver homogenates by 300% while likewise increasing HNE in adult liver by about 200% (data not shown). Marked increases in MDA levels were also seen in both adult (181%) and fetal (160%) tissues. Comparable responses were seen in isolated mitochondria, always with higher values in fetal liver mitochondria than in those from the adult tissue. When isolated mitochondria from adult and fetal liver were exposed to another prooxidant (t-butyl hydroperoxide) *in vitro*, mitochondria from fetal liver generated 3.5 times more HNE than did those from adult liver (data not shown). Since the three enzyme systems primarily involved in the metabolism of HNE are lower in fetal tissues than in adult, we investigated the possibility that low metabolic elimination of HNE in fetal liver is connected to the higher steady-state levels of the aldehyde the fetal tissue.

4.3.4. Adult and fetal differences in metabolism of HNE

The metabolism of HNE occurs primarily (but possibly not entirely) by three enzymatic systems, aldehyde dehydrogenase (ALDH), glutathione S-transferase (GST) and alcohol dehydrogenase (ADH) (figure 7). This concept has been based on the identification of primary products of these three reactions, 4-hydroxy-2-nonenoic acid, for ALDH, 1,4-dihydroxy-2-nonene, for ADH and a glutathione-HNE-conjugate for the GST-catalyzed reaction (68,88,89).

In our laboratory, using inhibitors of these three enzyme systems, we confirmed that in fetal and adult liver, these pathways account for over 90% of HNE catabolism. This is illustrated in figure 8 where 75 nmoles of HNE were added to one ml of adult or fetal liver homogenate and HNE remaining after various time intervals (5 minutes for this figure) was determined. Each enzyme system was driven by addition of specific cofactors for the system, NAD for ALDH, NADH for ADH, and GSH for GST and the enzyme inhibitors added were disulfiram (DIS) for ALDH, pyrazole (PYR) for ADH, and ethacrynic acid (E.A.) for GST. When all 3 inhibitors were included in the incubation, only 5% or less of the added HNE was metabolized (bars to the far right of figure 8). The lower capacity of these systems for HNE metabolism in the fetal liver is apparent from comparison of the bars for fetal and adult samples (open vs hatched) representing samples containing no inhibitors. For adult liver, over 90 % of all HNE was metabolized within the 5 minute incubation while for fetal liver homogenates, this value was 47%, 39% and 85% for incubations supplemented with NAD (ADH), NADH (ALDH), or GSH (GST), respectively. This adult/fetal difference was confirmed by time courses which quantitated net metabolism in both whole liver homogenates and in isolated mitochondria (data not shown but submitted to Hepatology for publication). Additionally, in fetal liver homogenates pyrazole had little effect on HNE metabolism indicating a low activity of ADH towards this substrate, while the striking inhibitory response to E.A. suggested a major role for HNE conjugation with glutathione. Thus, these studies imply that low metabolism of HNE may be one mechanism underlying its higher accumulation in fetal liver than in adult and additional

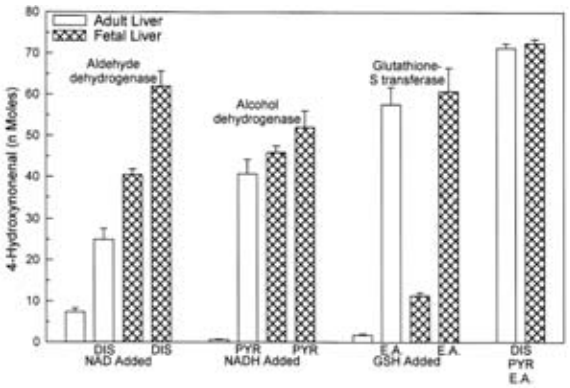

Figure 8. Metabolism of 4-hydroxynonenal by adult and fetal liver homogenates. Dams (Sprague Dawley rats) were sacrificed on gestation day 19. 75 n moles of 4-hydroxynonenal were added to one ml volumes of homogenates of adult or fetal liver. The reaction was terminated by addition of acetonitrile/acetic acid (24/1) five minutes after initiation of the reaction and the remaining 4-hydroxynonenal determined. Cofactors (noted under the x axis) for the reactions (noted above the corresponding bars) were added to each reaction; 1.0 mMolar for NAD and NADH and 100 uMolar for GSH. Inhibitors for the specific reactions were disulfiram (DIE), pyrazole (PER), and ethacrynic acid (E.G.). The data points are means +/- SEM., n= 6 adult livers and 6 sets of fetal livers from 6 pregnancies.

experiments (90) suggest that fetal liver mitochondria may be more prone to HNE formation than the organelle from adult liver under identical oxidant stress conditions.

5. SUMMARY

Generation of oxygen free radicals is a given component of aerobic life, however the elegant interactive detoxifications present in tissues are generally sufficient to prevent significant damage. The fetus, on the other hand, represents a system which may possess extraordinary sensitivity to oxidative stress. It is a body of rapidly replicating and differentiating (mostly in the embryonic stage) cells, a setting which places high demand on metabolic energy production. These are the very processes which generate the electron fluxes which produce reactive oxygen species. Additionally, fetal tissues contain higher levels of iron and copper than the adult yet they have lower levels of non-enzymatic and enzymatic detoxification systems needed to blunt the oxidative assault. The data presented here suggest that low antioxidant defenses may be an important factor that predisposes these tissues to oxidative stress. Studies in our laboratory have provided a variety of evidence that ethanol can produce oxidative stress in cultured fetal cells and in fetal brain and liver following short-term maternal ethanol consumption. In cultured fetal rat hepatocytes, oxidant-related damage caused by ethanol exposure can be mitigated by augmenting/normalizing antioxidant defenses. The only antioxidant system that appears to be compromised by ethanol is GSH stores, however this is only slight in the *in vivo* setting. An additional factor contributing to ethanol-related damage could be production of reactive aldehyde products of lipid peroxidation. *In vivo* exposure to ethanol strikingly enhances HNE content of fetal liver and two underlying mechanisms for this may be low metabolism of this toxic aldehyde and a predisposition to its formation.

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