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Is the use of alcohol in the present time common and socially accepted? In society at large, bars are widespread and frequently visited by the populace. Social clubs, arenas where young people gather on weekends and meet new friends, are usually centered around the presence of alcohol. Business deals cemented over lunch or dinner are commonly accompanied by alcoholic beverages. Advertisements, whether on radio, television, or billboards, give testimony as to how the consumption of a particular alcoholic beverage with a meal would enhance the meal. There is no Super Bowl Game that is not associated with multiple prominent beer commercials. In numerous religions there are many rites and special occasions that mandate the use of wine. Thus, the use of alcohol in our society is clearly socially acceptable.

One might inquire, why ask such a question with an answer that is so self evident? When one starts to peruse the detrimental effects of alcohol, this question and its answer become paradoxical. Alcohol use can lead to dependency (Core Consumer Resources). Alcoholism is defined as "a chronic disorder characterized by dependence on alcohol, repeated excessive use of alcoholic beverages, development of withdrawal symptoms on reducing or ceasing alcohol intake, morbidity that may include cirrhosis of the liver, and decreased ability to function socially and vocationally" (Google; Philpot). The National Institute of Alcohol Abuse and Alcoholism (NIAAA) reports that the incidence of adults who visit a physician and have an alcohol problem is at least 20 percent (NIAAA, *Screening for Alcoholism*). Furthermore, the chronic use of alcohol is the second most frequent cause that of severe liver disease which requires liver transplants (Anantharaju and Van Thiel 257-268). In addition, it has been reported that four out of every ten automobile accidents are attributed to the use of alcohol (Pusat). In fact, the teratogenic effects of alcohol are so well recognized by the medical and governing authorities that the law requires a warning label to be placed on all alcoholic beverages that are sold in the United States. This warning states, "Government Warning: (1) according to the surgeon general women should not drink alcohol during pregnancy because of the risk of birth defects. (2) Consumption of alcohol beverages impairs your ability to drive a car or operate machinery; it may cause health problems (*On the back of any alcoholic beverage sold in America.*)." Faced with the detrimental effects of alcohol, such as those outlined above, some people may question whether drinking alcohol should be as socially acceptable.

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Most people are familiar with the severe criminal penalties associated with drinking and driving, and there are public safety messages that address this issue. But what about the surgeon general's first warning: drinking during pregnancy? Many women do not even know that they are pregnant during the first few critical weeks of fetal development. Unknowingly, these women consume alcohol, which is then absorbed by the developing fetus. In addition, there are women who know that they are pregnant and erroneously conclude that one drink at a party will not hurt their fetus. The paper will attempt to give its readers an understanding of the effects alcohol has on fetuses during pregnancy and to review one mechanism via which these detrimental effects occur.

Prior to the 1960's, it was believed by the medical community at large that the placenta acted as a protective barrier to all environmental agents which might harm the fetus. However, in the 1960's, many disfigured children with shortened or missing limbs were born to mothers who took the drug thalidomide to reduce nausea during their pregnancies. Several reports were published detailing the link between thalidomide and these birth defects. This opened the field of research into teratogenic agents, one of which is alcohol.

In order for an agent to be considered teratogenic it must meet the following criteria: "The agent must cause death, malformations, growth retardation, and/or functional disorders (Randall 554-561)"; the effect must be dose related, i.e. a larger dose will result in greater damage; there must be critical period for susceptibility; and last, the vulnerability to the agent must be affected by interaction of genetic and environmental factors. Early studies on animal models with ethanol proved that ethanol is teratogenic. Ethanol is the type of alcohol primarily used in alcoholic beverages. For example, an experiment was performed by Carrie L. Randall and colleagues in 1977 on pregnant C57BL/6J mice, a strain of mice that prefers alcohol in a choice situation with water (It should be noted that most rodents will avoid drinking water if it contains alcohol.) (Randall 554-561). Pregnant mice were exposed to alcohol during (the human equivalency of) gestational days 5-10. The results showed that the alcohol-fed group produced more pups with malformations than the controlled group; larger doses of alcohol detrimentally affected the pup weight and rate of malformation (Fig. 1). The study also demonstrated that when the pregnant mice were exposed to

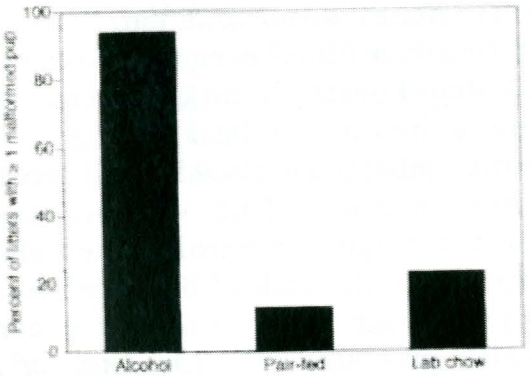

FIG. 1. Results from an early animal study conducted to establish the teratogenicity of alcohol (Randall et al. 1977). C57BL mice were exposed to 35% ethanol-derived calories (vs. sucrose-matched calories or lab chow) from GD 5-10. Statistical tests showed more malformed fetuses in the ethanol-treated group.

alcohol on Gestational Days 5-10, the pups were born with anomalous kidney and limb development. In addition, other studies showed that exposure to alcohol on Gestational Day 7 resulted in facial anomalies similar to those seen in children with Fetal Alcohol Syndrome. Research performed on the brain and its development showed that during different critical periods of growth, alcohol is teratogenic on specific cell population of the brain. Finally, several studies have shown that different species of mice are more vulnerable to alcohol-induced birth defects than others (Randall 554-561).

The term Fetal Alcohol Syndrome (FAS) was initially coined to describe a pattern of birth defects found in children born to mothers who consumed alcohol during their pregnancies (NIAAA, *Fetal Alcohol Exposure*). The first patients were diagnosed with FAS because of their common anomalous facial features. The common features noted were microcephaly, abnormally small development of the head; short palpebral fissures, the gap between the upper and lower eye lids; a hypoplastic (underdeveloped) philtrum, which leads to the appearance of a large gap between the nose and the mouth; a thin upper lip; and, in infancy, retrognathism (a condition where one or both jaws are posterior to their normal position (Farlex[10]) (Sulik 366-375). The NIAA defines FAS by four criteria. First, the mother must have ingested alcohol during her pregnancy. Second, the child must have the characteristic facial abnormalities for FAS as described above. Third, the child must have some growth retardation, and four, there must be brain damage to the child (NIAAA, *Fetal Alcohol Exposure).* A diagnosis of FAS will only be made if all four of the FAS criteria are met. Children with FAS may also have abnormalities such as cleft palates, high arched palates, congenital heart defects, epicanthic folds, hypetelorism (widely spaced eyes), atypical seizures and endocrinopathies. However, these conditions are not specific for FAS and therefore are not used when diagnosing patients with FAS (Sulik 366-375).

The most devastating consequences, and the most obvious signs of prenatal alcohol exposure, are those related to changes in the brain and the associated behavioral and cognitive impairments. The most obvious and consistent finding among alcohol-exposed patients is the overall smaller cranial vault and reduced brain size. Different techniques utilised for viewing living brains, such as magnetic resonance imaging (MRI), used on FAS patients have shown common reductions in size of specific brain structures (Figure 2) (Chirstensen 28). Studies have shown that alcohol affects the gray and white matter in different ways (Riley and McGee 357-365).

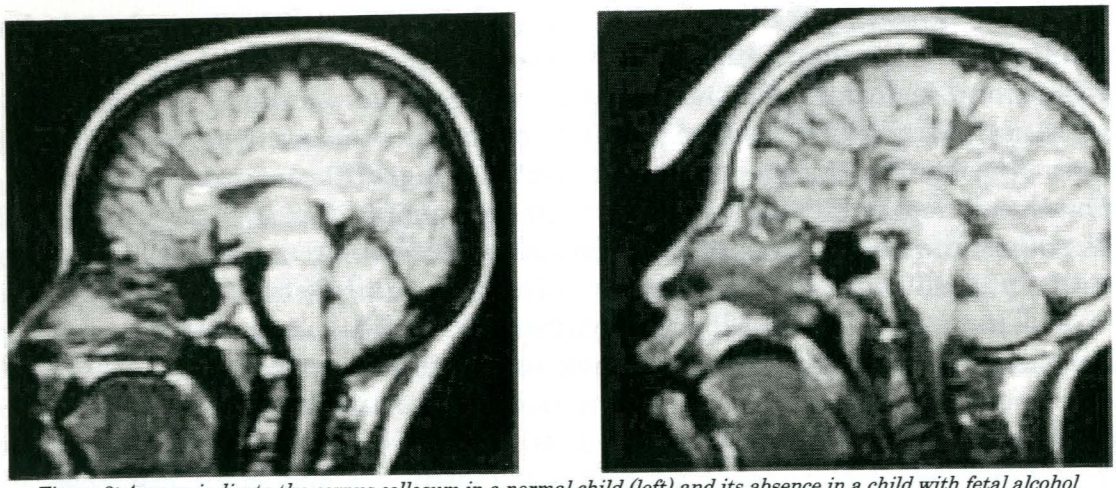

Figure 2: Arrows indicate the corpus collosum in a normal child (left) and its absence in a child with fetal alcohol syndrome (right).

When compared to controlled groups in a study, patient exposed to alcohol had a relative increase in gray matter and a decrease in white matter. Therefore, white matter hypoplasia was more significant than gray matter. One of the brain structures that are reduced in size are the basal ganglia, specialized nerve cell clusters located deep in the cerebral hemispheres and in the upper brain stem. The basal ganglia assist in initiating and regulating movement. Research found that patients with damaged basal ganglia had impaired spatial memory, impaired set shifting and a range of impaired cognitive processes (NIAAA, *Fetal Alcohol Exposure*; Ravizza and Ciranni 472-483). The cerebellum, which is involved in balance, gait, coordination, and cognition, is also commonly found to be reduced in size. Lastly, a major effect of prenatal alcohol exposure is the impaired development or complete absence of the corpus callosum, which is a large group of nerve fibers that allow communication between the right and left hemispheres of the brain. In addition, the corpus callosum was found to be significantly displaced in three-dimensional space. An estimate of seven percent of patients diagnosed with FAS lack a corpus callosum (NIAAA, *Fetal Alcohol Exposure*; Riley and McGee 357-365). There has been an increasing amount of evidence that the loss of neurons, and thereby the defects of these brain structures, may be mediated by apoptosis (as will be discussed further in the paper) (Ramachandran et al. *Ethanol-induced oxidative stress, 577-588*).

There is a wide range of neuropsychological deficits related to prenatal alcohol exposure. Impairment of overall intellectual performance, memory, language, attention, reaction time, visuospatial abilities, executive functioning, fine and gross motor skills, and social and adaptive functioning are examples of some of the above deficits. This is possibly the reason that the effects of prenatal alcohol exposure on the intelligent quotient (IQ) have received a lot of attention. Nevertheless, most people with FAS are not mentally retarded. Approximately 25% of people diagnosed with FAS have an IQ score less than 70 (IQ scores range approximately between 20 and 140).

It is estimated that the average IQ score for people with FAS is between 65 and 70 (mild mental retardation is recorded to be between 55 and 70). Researchers have found that the IQ of a FAS patient decreases in proportion to the increase of facial anomalies (Riley and McGee 357-365). Children with FAS were found to have an impaired ability in the initial stages of memory formation, yet, had the normal ability to recall information previously learned. This characteristic becomes clinically important when diagnosing a child with FAS because it rules out Down's syndrome, in which recall is also impaired along with the actual learning of information itself.

Another clinically important and commonly misdiagnosed aspect of FAS is the fact that children prenataly exposed to alcohol exhibit attention disorders and are diagnosed with attention-deficit/hyperactivity disorder (ADHD) (NIAAA, *Fetal Alcohol Exposure*). Although inattention is present in both children with FAS and ADHD, impulsivity is more specific for children with ADHD. Additionally, children with FAS have deficits in visual attention, while auditory attention deficits were only present when the intertarget intervals were long (Riley and McGee 357-365) . Furthermore, they displayed difficulty in set shifting, shifting attention form one assignment to another, which may attribute to their problems with attention (NIAAA, *Fetal Alcohol Exposure*). Damage to the basal ganglia may be a cause of these symptoms. Dysfunctions in fine-motor skills, including tremors, weak grasp, poor hand coordination, and impairments in balance are found in children with FAS and are attributed to damage to the cerebellum (Riley and McGee 357-365).

The range of birth defects of children born to mothers who drank alcohol during pregnancy varies greatly because of the multitude of different variables that can play a role in affecting fetal development. The defects a child is born with depend on the timing of the exposure, i.e. during which stage of fetal development the mother drank alcohol; how much alcohol the mother drank; genetic factors in both the mother and the fetus with regard to the metabolism and functional sensitivity toward alcohol; nutritional factors influencing via blood glucose levels or other means how much alcohol reaches the fetus; if there was any other teratogenic agent taken at the same time, i.e. cocaine, marijuana, nicotine [8], and the age of the mother. This not only increases the complexity of diagnosing FAS, but it also increases the complexity of the research done on the effects of alcohol on the fetus. It is not unexpected, then, to see that babies born to two different mothers who binged on the same amount of alcohol have different deficiencies. Where one might be severely affected by the alcohol exposure, the other may have no apparent defects. Because the effects of prenatal alcohol exposure "lie on a continuum or present a spectrum of disorders" (Riley and McGee 357-365), the term fetal alcohol spectrum disorder (FASD) was coined as an all encompassing term to describe the range of effects that can result in people whose mothers drank alcohol during pregnancy. As noted above, in order to diagnose a patient with FAS, certain distinct facial features must be present. However, many

children who are exposed to alcohol in-utero are not born with the facial features of FAS, yet display many of its other characteristics. Since all four of the FAS characteristics are not present, these children would fall under the description of FASD. Children with FASDs have similar defects to those children diagnosed with FAS, yet the defects are usually found to be less extreme.

"The paradox of alcohol is that, although its effects on the developing organism are fairly specific (i.e., heavy prenatal alcohol exposure produces an identifiable syndrome, and certain brain regions are affected whereas others are not), it is also a ubiquitous drug that affects many physiologic systems (Randall 554-561)." The multiple interactions that ethanol is known to have with a myriad of target molecules makes it difficult to discern the exact loci of its actions. Furthermore, these interactions can be dynamic: ethanol can both interact and/or functionally alter target molecules that might affect the original interaction, thereby making specific dose effect relationships complicated. When studying the developing brain, it becomes even more complex because of the genetically regulated continual changes in structure and cell biologic cascades that occur during brain development. There are a large number of mechanisms that have been identified as possible methods responsible for the range of FASD. Yet, a few mechanisms have been substantially supported via experimental studies that concentrated on ethanol's specific molecular interaction with target tissues. Examples of some of these mechanisms are: oxidative stress; altered glucose transport and utilization; suppression of DNA synthesis; impaired neurogenesis and gliogenesis; mistimed events of cell generation, migration, neurite outgrowth, synaptogenesis, and myelination; altered cell cycle and altered regulation of gene expression, interference in cell signaling pathways (Goodlett et al. 394-406). This paper focuses on oxidative stress and the mechanism by which it affects the fetal brain.

The excessive production of free radicals by the body can result in a state called oxidative stress. It has been suggested that oxidative stress plays a main role in many pathways of alcohol-induced damage. Reactive oxygen species (ROS), a class of free radicals that contain oxygen, are of particular importance. Essential complex molecules can be damaged or completely destroyed (peroxidized) via ROS; i.e. lipid molecules, proteins, DNA. It has been demonstrated in experiments that alcohol can increase the production of ROS and enhance peroxidation of lipids, proteins and DNA (Wu and Cederbaum 277-284).

Free radicals are highly unstable molecules because of the unequal distribution of electrons within the molecule. Consequently, free radicals are extremely reactive in their attempt to create a stable compound. To reach this stable state, free radicals can undergo a few different chemical reactions. First, they may undergo hydrogen abstraction, by which the free radicals interact with another molecule that can donate a hydrogen molecule and bind

to the hydrogen, converting the hydrogen donor to a new free radical. The second reaction it can undergo is addition. In this reaction, the free radical binds to a stable molecule, converting the newly formed combined molecule to a free radical. Additionally, the free radical can undergo termination, in which two free radicals react with each other forming a stable molecule. Last, they may undergo disproportionantion, a reaction by which two of the same free radicals react with each other, one donating an electron to the other, so that two different stable molecules are formed (Wu and. Cederbaum 277-284).

Oxygen is regularly involved in free radical formation. Oxygen is extremely important for cell function because it plays a central role in a series of biochemical reactions in the respiratory chain. The respiratory chain takes place in the mitochondria and provides the energy needed for the cell to carry out its functions and reactions in the form of adenosine triphosphate (ATP). In the respiratory chain, an electron is transferred from reduced nicotinamide adenine dinucleotide (NADH) to the first component of the respiratory chain and a proton (H^+) is released into the surrounding fluid. The reduced component of the respiratory chain passes the electron on to other molecules in the chain until it is finally transferred to molecular oxygen (O_2). O_2 then reacts with the protons to produce water. In this reaction, O_2 can receive a total of four electrons, one at a time, and an equal number of protons to produce two molecules of water. It is during this reaction that a variety of oxygen radicals are formed as intermediate products. These include: superoxide ($O_2 \cdot^-$); peroxide ($O_2^=$), which usually undergoes a quick reversible reaction and exists as hydrogen peroxide (H_2O_2) in cells; and the hydroxide radical ($\cdot OH$). These radicals are considered to be the primary ROS. Although, only about two to three percent of O_2 consumed in the respiratory chain is converted to ROS, the reduction of O_2 to ROS is thought to be the primary mechanism by which oxygen is toxic in biological systems (Wu and Cederbaum 277-284).

There are several cellular mechanisms that prevent the formation of, or act to, detoxify the ROS. These mechanisms use molecules called antioxidants to protect the cell against ROS. In the presence of alcohol, ROS production is enhanced and antioxidant levels or activity is reduced. The imbalance between ROS production and degradation and the repair of damaged complex molecules is known as oxidative stress (Wu and Cederbaum 277-284). Oxidative stress is associated with a number of neurodegenerative diseases and ethanol-induced toxicity of a number of organ systems, including the liver and the brain (Goodlett et al. 394-406).

There are many processes that are involved in causing alcohol-induced oxidative stress. One such mechanism is the change in NAD+/NADH ratio in the cell. This change results from alcohol metabolism, which is a two step process. First, alcohol is converted to a toxic and reactive molecule, acetaldehyde, via the enzyme alcohol dehydrogenase. Then, acetaldehyde is

converted to acetate via the enzyme aldehyde dehydrogenase. These reactions result in the formation of one NADH per reaction, which provides more starting material and thereby increasing the activity of the respiratory chain. This results in an increased use of O_2 and ROS formation. The production of acetaldehyde in the first step of alcohol metabolism is another mechanism by which damage can occur. Radical formation can occur with the interactions of acetaldehyde with proteins and lipids and can lead to cell damage. The increase in ROS can damage the mitochondria and result in a decrease in ATP production. Cell structure is also affected by alcohol's interaction with lipids, enzymes and proteins of the cell (i.e. the cell membrane contains phospholipids, phosphates containing lipids, which is peroxidized via ROS). Another mechanism is that alcohol can induce hypoxia in tissues, because more oxygen is required to metabolize the alcohol. In addition, alcohol has an effect on the immune system, where there is a change in the production of cytokines, signal molecules, thereby activating a group of biochemical processes. Furthermore, alcohol increases the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which metabolizes alcohol and other molecules producing ROS in its process. Alcohol also increases the level of iron in the cell, which promotes the generation of ROS. Other factors involved in alcohol-induced oxidative stress are the fact that antioxidants and other molecules, such as glutathione (GSH), are affected by alcohol; biochemical reactions form a radical from alcohol; and alcohol promotes the conversion the enzyme xanthine dehydrogenase into xanthine oxidase, which can form ROS (Wu and Cederbaum 277-284).

The major system that produces ROS is the respiratory chain in the mitochondria. Because the mitochondria use 80 to 90 percent of the oxygen consumed in the body, even though only a small amount of ROS is produced it still generated most of the ROS in the body. Another main source of ROS is cytochrome P450 mixed-function oxidases, which are group of iron containing enzymes. A large variation exists among these enzymes, some of which have the responsibility to remove toxic compounds present in the cellular environment and in the food ingested, including alcohol. ROS is generated in small amounts via the use of oxygen in the biochemical reactions catalyzed by cytochrome P450. CYP2E1 is particularly active in generating ROS. When studying alcohol-induced oxidative stress this enzyme is of particular interest, because not only is it known for metabolizing alcohol, but it increases in activity after alcohol exposure (Wu and Cederbaum 277-284).

The most reactive ROS is the hydroxyl radical (·OH). Usually, superoxide produced by the cells dismutates into hydrogen peroxide and oxygen. Hydrogen peroxide is usually removed via catalase. However, during states of oxidative stress more superoxide and hydrogen peroxide are produced and overloads the cells ability to eliminate these radicals. The hydroxyl radical is generated mostly through reactions with free metals, specifically free iron and copper ions. Superoxide plays a key role in this

process. This is a two step process. Superoxide reacts with metal and puts the metal in a reduced state. Hydrogen peroxide (H_2O_2) produces the hydroxyl radical by removing an electron from a free metal. Subsequently, the superoxide radical reacts with the metal to regenerate it so that it will be available for another reaction with hydrogen peroxide. Studies have shown that alcohol raises the amount of free iron in the body. Not only with iron-rich alcohol, but also because chronic alcohol consumption enhances iron absorption from food (Wu and Cederbaum 277-284).

Once the hydroxyl radical is formed it can react with carbohydrates, proteins, lipids, and nucleic acids to form peroxyl radicals (ROO·). These intermediates can react with cellular macromolecules and cause damage or death to the cell. The building blocks of proteins are amino acids. Each amino acid has a different sensitivity towards interactions with ROS. For instance, the amino acids cysteine, methionine, and histidine are more prone to oxidation by the hydroxyl radical. Consequently, enzymes that have these amino acids located at an active site on the enzyme can become inactivated when exposed to ROS. Additionally, the oxidation of the protein can cause changes in its three-dimensional structure, fragmentation, aggregation and cross linking. This can lead to protein degradation by a cellular mechanism that recognizes damaged protein and eliminates it from the cell as waste byproduct (Wu and Cederbaum 277-284).

Phospholipids are essential components of all membranes. Not only do they surround cells but subcellular structures as well, such as nuclei and mitochondria. They are composed of lipids and phosphate groups plus other components which modify their structure and function. Accordingly, damage to phospholipids can compromise subcellular function as well as cellular viability. Lipids are comprised of polyunsaturated fatty acids or saturated fatty acids. The double bonds between two adjacent carbons found in unsaturated fatty acids are particularly sensitive foci for reacting with ROS. Lipid peroxidation, the reaction between ROS and polyunsaturated fatty acids, is one of the hallmarks of oxidative damage (Wu and Cederbaum 277-284). For example, in-utero ethanol exposure increases lipid peroxidation in mitochondria as seen by increases in mitochondria 4-hydroxynonenal (a toxic product of lipid peroxidation) (Figure 3) (Ramachandran et al., *In utero ethanol exposure,* 862-871).

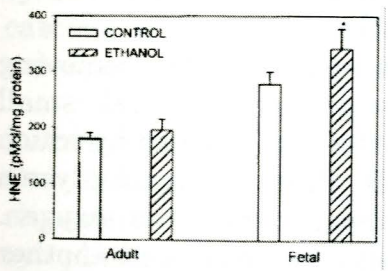

Fig.3. In utero ethanol exposure increases HNE content of fetal brain mitochondria. HNE produced in maternal and fetal brain mitochondria after a 2-day binge ethanol exposure was measured by high-performance liquid chromatography and expressed in pmol/mg protein. Values are mean ± SEM. *$p \leq 0.05$ compared with control values.

Damage to the DNA of a cell can cause mutations in the proteins that it codes for. These mutations can alter the structure of the protein, thereby causing changes in its function or complete inactivation. ROS causes breaks in the strands of DNA, removal of nucleotides from the DNA sequence, and/or

modification of the organic bases of the nucleotides. This is a major cause of mutations in DNA. Even though the cell has mechanisms to repair DNA damage, when there is excess damage caused by ROS permanent damage may occur causing detrimental effects to the cell (Wu and Cederbaum 277-284). Ethanol has been shown to increase DNA damage as assessed by DNA fragmentation (Figure 4) (Ramachandran et al., *In utero ethanol exposure,* 862-871).

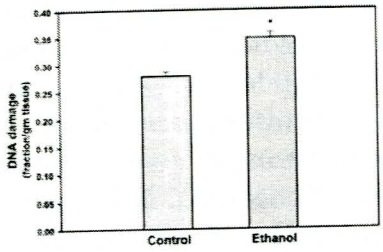

Fig.4 In utero ethanol exposure increases DNA fragmentation in the fetal brain. DNA fragmentation was measured in control and ethanol-exposed fetal brain by a fluorometric method with the dye Hoechst 33258. The experiment was performed as described in "Methods." Values are expressed as mean ± SEM for $n = 3$ as fraction of DNA damage per gram of tissue. *p 0.05 compared with control values.

The production of ROS is a natural process in the body, and there are mechanisms by which the body can remove them before they cause damage. These mechanisms include both nonenzymatic and enzymatic processes. Chronic exposure to alcohol may impair some of these mechanisms, leading to further damage of an organ. Superoxide dismutases (SODs), catalase, and the glutathione peroxidase system are enzymes involved in the removal of ROS. There are several different types of superoxide dismutases located in different part of the cell. Yet, they all function to catalyze a reaction that removes the superoxide radicals from the cell before they could do any damage. There have been controversial studies of the effects of chronic alcohol exposure on the activity and cellular content of superoxide dismutases. Studies using models that injected the alcohol directly into the intestine of the laboratory animal found that there was a decrease in superoxide dismutases (Wu and Cederbaum 277-284).

Catalase and glutathione peroxidase are both responsible for removing hydrogen peroxide. Catalase is found mainly in peroxisomes, small membrane bound components in the cell. There are two mechanism by which catalase can remove hydrogen peroxide from the cell. First, it may catalyze a reaction between two hydrogen peroxides producing water and oxygen. Secondly, it may catalyze a reaction between hydrogen peroxide and another molecule that is a hydrogen donor. This reaction produces one water molecule and an oxidized donor molecule. The glutathione peroxidase system requires the enzymes glutathione peroxidase and glutathione reductase, in addition to two cofactors glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) to complete its reaction to remove hydrogen peroxide.

Glutathione not only serves as a cofactor for a few enzymes, but it can also react with ROS on its own to detoxify them (i.e. the hydroxyl radical). Glutathione is probably the most important antioxidant in the body due to its multiple functions. Hence, the enzymes that facilitate the production of glutathione are essential for the protection of the body from oxidative stress. Research on alcohol has shown that it reduces the levels of glutathione in the

cell, especially in the mitochondria where there is a large number of glutathione present to eliminate the ROS produced in the respiratory chain. The mitochondria do not have the ability to generate glutathione, thus it is transported into the mitochondria via a carrier protein. Studies have shown that the depletion of glutathione in the mitochondria is a result of alcohol's effect on the function of the carrier protein.
Other non-enzymatic antioxidants include vitamin E (α-tocopherol), vitamin C (ascorbate), and ß-carotene. (Wu and Cederbaum 277-284)

Oxidative stress can initiate a cascade of events in neurons that lead to apoptosis. Studies have shown that oxidative stress due to ethanol exposure causes damage to the mitochondria, which then release proapoptotic molecules that induce apoptosis (Ramachandran et al., *In utero ethanol exposure,* 862-871). Apoptosis, programmed cell death, is a controlled mechanism by which specific cellular pathways mediate cell death. The apoptotic death is recognizable by the break up of the nuclear envelope, nuclear fragmentation, changes of the cell morphology (i.e. blebbing of the cell membrane), and cell fragmentation. A key component to apoptosis is the presence of biochemical changes within the cell and its surrounding environment. In some cells, phosphatidylserine appears on the cell membrane's extracellular leaflet, which signals phagocytotic cells to engulf the apoptotic body. In addition, there is an increase in cytoplasmic levels of proapoptotic proteins that are released from the mitochondria, and an increased activity of caspases, a family of cysteine proteases that cleave specific targets that destroy cellular structures (i.e. nuclear lamina, cytoskeleton, and DNA). There are two pathways that could start the cascade of events that leads to apoptosis, the intrinsic pathway and the extrinsic pathway.

In the intrinsic pathway, the release of cytochrome C and apoptosis inducing factor from the mitochondria is a major step that leads to apoptosis. Once released from the mitochondria, cytochrome C binds to dATP to form an oligomeric complex with an apoptosis-activating factor (Apaf-1) in the cytoplasm. This complex activates the initiator caspase-9, which then activates effector caspases 3,6, and 7 "in a positive amplification loop which leads to apoptosis" (Ramachandran et al., *Ethanol-induced oxidative stress 577-588*). Apoptosis inducing factor can amplify the cytochrome C/caspase-9/caspase-3 pathway as well as "cause a caspase independent nuclear apoptosis (Ramachandran et al., *In utero ethanol exposure,* 862-871)" Apoptosis can also be activated from the outside of the cell, i.e. the extrinsic pathway. A killer lymphocyte that has a ligand on its outer surface, such as Fas or TNF, recognizes a protein located on the outer surface of a target cell, such as Fas (CD95) or TNF-R. The interaction between the ligand and the protein cause the assembly and aggregation of an adapter protein and procaspase-8. Procaspase-8 is then cleaved and becomes activated caspase-8 which starts the caspase cascade and finally apoptosis.

The developing brain is in the process of generating many new neurons and neuronal pathways, yet only the ones that reach their targets during development are stimulated to continue growing via growth factors secreted from the target areas. Some of these cells are temporary: more than fifty percent of the original neurons undergo apoptosis. Therefore in utero, when the brain is developing, various nerve cells may already be primed for apoptosis. Consequently, putting the cells in an environment of a potentially proapoptotic compound such as alcohol can induce an exaggerated apoptotic response(Ramachandran et al. *Ethanol-induced oxidative stress, 577-588*).

ROS may either be the first step in the cascade of events that commits the cell to programmed death and/or they may play a role in signaling or amplified the cascades of apoptosis (Ramachandran et al., *Ethanol-induced oxidative stress 577-588*). In a state of oxidative stress 4-

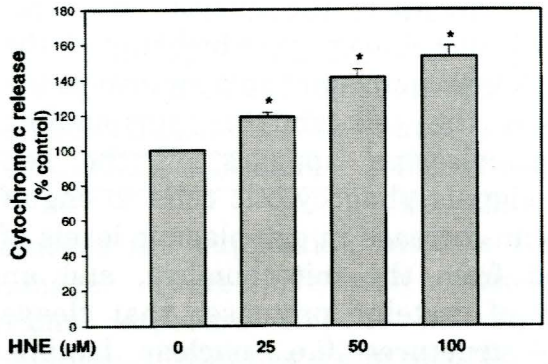

Fig.5 HNE induces cytochrome *c* release from fetal brain mitochondria assessed by Western blotting. Densitometric measurement of bands representing cytochrome *c* release from three independent experiments. Values are mean ± SEM. $*p \leq 0.05$ compared with control values with no HNE exposure.

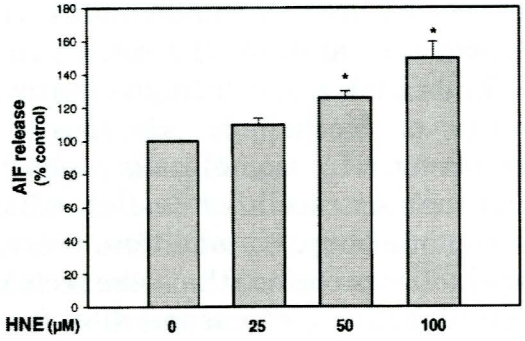

Fig. 6. HNE causes release of AIF from fetal brain mitochondria by Western blotting. Densitometric measurement of bands representing AIF release from three independent experiments $*p \leq 0.05$ compared with control values with no HNE exposure.

hydroxynonenal, a product of lipid peroxidation, increases in the cell (Figure 3). 4-hydroxynonenal was found to stimulate cytochrome C and apoptosis inducing factor (AIF) to be released from the mitochondria, and activate caspase-3 (Figures 5 & 6). In addition, 4-hydroxynonenal has been found to inhibit multiple enzymes, i.e. the Na+/K+/adenosine triphosphate and adenine nucleotide translocator, an enzyme required for mitochondrial functioning. Accordingly, 4-hydroxynonenal can induce death in cerebellar granule cells, PC12 cells, and cultured hippocampal neurons. In a study done by Ramachandran and colleagues determined that 4-hydroxynonenal's effects on the mitochondria were similar to apoptosis related effects on mitochondria caused by in-utero exposure to ethanol. Mitochondria were isolated from control and ethanol exposed fetal brains at gestational day 19. Using the amount of 4-hydroxynonenal present in the lipid environment of the mitochondria during oxidative stress caused by ethanol, mitochondrial swelling and an increase in the release of cytochrome C and apoptosis releasing factor were observed in mitochondria isolated from control brains. From this they concluded that

perhaps the production of 4-hydroxynonenal in the mitochondria of a fetal brain is one mechanism by which ethanol brain damage occurs in the developing brain (Ramachandran et al., *In utero ethanol exposure,* 862-871).

Another study carried out by this group illustrated that when fetal cortical neurons were exposed to ethanol, the mitochondria exhibited a rapid increase in ROS and 4-hydroxynonenal, the start of the intrinsic pathway, and apoptosis. Using trypan blue exclusion to test for the cells viability, this in vitro study showed that ethanol significantly decreased cell viability. Because glutathione is known to help prevent oxidative stress, they treated some of the neurons with N-acetylcysteine (NAC), a potent antioxidant, to increase the levels of glutathione (Ramachandran et al., *Ethanol-induced oxidative stress 577-588*). This treatment helped decrease apoptosis in the cortical neurons (Figure 7). This research elicits the idea that perhaps the depletion of glutathione is one way by which ethanol induces oxidative stress.

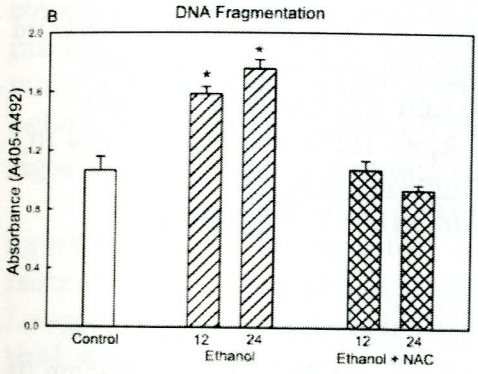

Fig. 7.. Enhancement of cellular GSH content prevents ethanol induced apoptotic cell death. As assessed by DNA fragmentation Results are expressed as percentage change in DNA fragmentation. Means (±SEM) represent six experimental values (*$P \leq .05$ compared with control with no NAC pretreatment).

In conclusion, the consumption of alcohol during pregnancy can have severe effect on fetal development. One of the most devastating outcomes is damage to the developing brain. This includes reduction in the size of the basal ganglia, cerebellum, and the corpus callosum. The alcohol present in most alcoholic beverages is Ethanol. Ethanol acts via many different mechanisms to induce such extensive damage. One such mechanism is oxidative stress. It has been implicated that the decrease in neurons in the fetal brain is due to apoptosis of the cells as a result of increased oxidative stress. Two possible ways by which ethanol causes oxidative stress are via an increase in the release of 4-hydroxynonenal from damaged mitochondria, and/or via the depletion of glutathione.

Because of the difficulty in identifying alcoholic pregnant women before they put their fetus in danger, much of the research now is focused on ways to intervene and prevent further damage or to cure damage that was already done. In order to find these strategies for intervention, the mechanisms of ethanol must first be identified. Some mechanisms have been identified, and interventions via these mechanisms are being studied. Yet, currently they have not found a possible way to identify the primary mechanism by which ethanol interacts with the developing brain tissue that initiates this pathogenesis cascade (Goodlett et al. 394-406).

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