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Brief Communication

OXIDATIVE STRESS AND THE FETOTOXICITY OF ALCOHOL CONSUMPTION DURING PREGNANCY

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Abstract—Pregnant Quackenbush Special mice were exposed to ethanol under semiacute (3.0 g/kg body weight intragastrically, days 7 to 12 of pregnancy), and chronic conditions (15% ethanol in drinking water for 5 weeks before and during pregnancy) to assess whether embryo-fetotoxic actions of the drug involve oxidative stress effects. Effects were monitored both in the maternal system and embryo. Alcohol compromised the maternal system by increasing the generation of lipid peroxides in the liver. It also decreased glutathione and vitamin E levels, and glutathione peroxidase and superoxide dismutase activities in this organ. Glutathione peroxidase activity in the maternal blood decreased. Only minor alcohol-induced changes occurred in the uterine endometrium, including decreased xanthine oxidase and increased γ-glutamyl transpeptidase. Similarly, only few changes were induced in day-12 embryos by alcohol. In this case, glutathione content and xanthine oxidase activity decreased while glutathione reductase activity increased following exposure to the chronic regime. With the possible exception of the maternal liver where evidence of oxidative damage was detected, these results do not reflect substantial changes in the antioxidant defences of either the pregnant mouse or embryo. However, the changes may contribute to the growth retarding and other fetotoxic effects of alcohol when they are totalled into the multifactorial actions of the drug.

Keywords—Ethanol, QS mice, Embryo-fetotoxicity, Antioxidants, Lipid peroxides, Oxidative stress, Fetal Alcohol Syndrome, Pregnancy, Free radicals

INTRODUCTION

Maternal alcohol consumption during pregnancy can produce embryo-toxic effects that, in humans, culminate in a variety of developmental anomalies designated as either Fetal Alcohol Effects or the Fetal Alcohol Syndrome (FAS).[1,2] There are a number of pharmacological mechanisms of ethanol that could impair development via either direct or indirect effects. Direct effects of alcohol, registered when the conceptus is exposed to the drug in vitro, include disturbances in glucose metabolism,[3] prostaglandin production,[4] cAMP-dependent protein kinases,[5] and DNA and protein metabolism.[6,7] Alternatively, ethanol may impair fetal development indirectly by interfering with the maternal capacity to support embryo-fetal development. In this context, disturbances in placental function and umbilical circulation,[8,9] glucose homeostasis,[10] temperature regulation,[6] uterine phospholipid methylation,[11] endocrinology,[12] and the availability of zinc[13] have been documented.

Ethanol toxicity in various somatic tissues of adult animals, and particularly the liver, can, in part, be attributed to an increased production of reactive oxygen species (ROS).[14,15] In hepatocytes, these potentially damaging ROS are generated, as a consequence of the metabolism of ethanol to acetaldehyde, mainly through the intervention of the ethanol-inducible cytochrome P450 (CYP2E1).[16] Various other mechanisms in these cells also generate ROS and free radicals from alcohol.[17] Xanthine oxidase (XO; E.C. 1.2.3.2), among other enzymes, can oxidize the acetaldehyde generated as a consequence of alcohol metabolism and, in so doing, can further promote the levels of ROS.[17]

Organisms have developed a variety of defenses, including enzymes and nonenzymatic antioxidants to protect themselves from ROS. The major endogenous

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antioxidant enzymes include, superoxide dismutase (SOD; E.C. 1.15.1.1), catalase (CAT; E.C. 1.11.1.6), selenium-dependent glutathione peroxidase (GSH-Px; E.C. 1.11.1.9), and glutathione reductase (GR; E.C. 1.6.4.2). The major nonenzymatic antioxidants include the reduced form of glutathione (GSH), vitamin C (ascorbic acid), and vitamin E (α-tocopherol). Glutathione is a vital component of the cell's antioxidant armory and affords protection from free radicals, ROS, and chemical electrophiles through its ability to interconvert between oxidized and reduced forms.[18] Extracellular glutathione is inactivated by the plasma membrane enzyme, γ-glutamyl transpeptidase (GGT; E.C. 2.3.2.2), which degrades the tripeptide to its constitutive amino acids.[19] Several of the above antioxidant enzymes also utilize glutathione as a cofactor in their catalytic activities.

Biomembranes contain substantial amounts of polyunsaturated fatty acids, which are highly susceptible to peroxidative breakdown. Lipid peroxidation is a free radical-mediated chain reaction that is enhanced as a consequence of oxidative stress and results in an oxidative deterioration of these membranous polyunsaturated fatty acids.[20] Vitamins E and C provide a major source of protection against the damaging effects of oxygen radicals and are of paramount importance in terminating peroxidative chain reactions of unsaturated lipids. Oxidative injury through lipid peroxidation will occur only when these protecting systems are insufficient to cope with the prooxidants that are generated.

It is not known with any certainty whether alcohol-induced oxidative stress presents a mechanism to explain the embryo-fetotoxic effects of the drug during pregnancy. In addition to faciliting the increased production of ROS in the liver as indicated above, alcohol consumption can lead to the diminishment of glutathione levels in this organ.[14,17] If glutathione were similarly depleted in the embryo-fetus during development, the conceptus could become more susceptible to the toxic effects of alcohol-induced ROS.[21] It has also been suggested that SOD may play a crucial role in protecting embryos against oxygen toxicity in vivo as well as in vitro.[22] In this context, superoxide radicals seem to play an important role in the process of blastocyst implantation, as well as in the synthesis of progesterone during early pregnancy.[23]

In view of these considerations, the present investigation was undertaken to determine whether alcohol consumption disturbs antioxidant defenses during pregnancy sufficient to account for either direct or indirect embryo-fetotoxic effects of the drug. We engaged the Quackenbush Special (QS) mouse as an animal model and exposed the animals to both semiacute and chronic regimes of alcohol known to induce developmental deficiencies and biochemical alterations.[3,10,11,24]

MATERIALS AND METHODS

Materials

Ethanol (Absolute, Analytical Reagent) was obtained from Rhone-Poulenc Laboratory Products (Clayton South, Victoria). Glycylglycine, γ-glutamyl-3-carboxy-4-nitroanilide (glucana), NADP, and catalase (106810) were purchased from Boehringer-Mannheim Australia Pty. Ltd. (Mount Waverly, Victoria). α-Tocopherol was obtained from Fluka Chemie (Switzerland) while 2,4-dinitrophenylhydrazine was purchased from Ajax Chemicals (Sydney, New South Wales). Ascorbic acid, glutathione (oxidised and reduced, GSSG and GSH, respectively), bathophenanthroline, NADPH, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT), xanthine, XO, GR, and aldehyde dehydrogenase were from Sigma Chemical Company (St. Louis, MO). Other chemicals were of analytical grade and experiments were carried out using demineralized and twice distilled water.

Animals and treatments

Female QS mice aged 7–12 weeks were used in all experiments and were housed under controlled environmental conditions (16 h light:8 h dark). The animals were permitted unlimited access to food and water. Pregnancy was brought about by pairing females with fertile males of the same strain. The females were examined for copulation plugs each morning, and the day of finding a plug was designated as day 1 or the first day of pregnancy.

In groups used to determine the semiacute effects of alcohol, mice were given 3.0 g/kg body weight of ethanol intragastrically[11] as a 25% (v/v) solution in distilled water using a 21-gauge mouse feeding needle for 6 d at 0800 h on days 7 to 12 of pregnancy. This treatment program coincides with the early stages of postimplantation and organogenesis when the embryos are most susceptible to a teratogenic insult and when alcohol induces defined metabolic and developmental alterations in mice.[3,10,11,24] Control animals were given isocaloric amounts of 34% (w/v) sucrose in place of alcohol.

In another group of animals used to determine the effects of chronic exposure to alcohol, mice were given 15% (v/v) ethanol in drinking water from 4 weeks before mating to day 12 of pregnancy.[25,26] The concentration of ethanol in the drinking water was gradually

increased by 1% per day over the first week in order to acclimatize the animals to ethanol. Thus, it was 8% (v/v) on the first day and 15% (v/v) on the eighth day of treatment. Animals in the control group were given alcohol-free drinking water. Water, ethanol, and food consumption were measured every day and energy intake was calculated for each animal in the control and treated groups.

Collection and preparation of tissues for assay

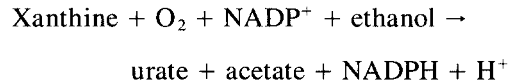
Control and alcohol-treated animals were killed at 1100 h on day 12 of pregnancy by cervical dislocation. Maternal blood samples were immediately collected by heart puncture, following which the liver, uterus, and embryos were excised through a midline abdominal incision and prepared for the assay of malondialdehyde (MDA), glutathione, GR, GSH-Px, SOD, XO, CAT, vitamin C, vitamin E, GGT, and protein. No attempt was made to isolate individual organs, such as the brain and liver, from the embryos for assay because their relatively small size impeded the preparation of contamination-free organs at a rate sufficient to unequivocally prevent introducing confounding manipulation-induced changes.

Blood samples were rapidly dispensed into 1.5 ml capacity Eppendorf tubes containing heparin and were held at 4°C until assayed. After excising the uterus, embryos were recovered by opening the organ beginning at the utero-tubal junction and proceeding to the cervical end along the antimesometrial axis. The endometrium was then gently recovered by scraping with a 22-gauge scalpel blade. The myometrium was discarded. All tissue samples, involving either the maternal liver, uterine endometrium or embryos, were homogenized at 4°C in 5 to 10 vol of buffered saline solution appropriate for the various assays. The homogenates were used without centrifugation in lipid peroxidation, CAT, glutathione, and vitamin E assays, but were otherwise centrifuged (600–3,500 × *g*, as appropriate, for 10 min at 4°C) to provide clear supernatants for the assay of all other enzymes and vitamin C. For assays undertaken on blood samples, whole blood was used in all cases, except for lipid peroxidation, which involved blood plasma only, and SOD, which required sedimented erythrocytes only.

Assay of lipid peroxides, enzymes, and antioxidants

Assays of lipid peroxides, enzymes, and antioxidants were conducted using spectrophotometric procedures and were performed in duplicate. Lipid peroxides, present as either MDA-like materials or MDA, were determined in blood plasma and tissue homogenates by the TBA reaction using the methods of Santos et al.[27] and Ohkawa et al.,[28] respectively.

XO catalyses the conversion of xanthine to uric acid. In the assay of this enzyme, coupled reactions involving CAT and aldehyde dehydrogenase were included to facilitate the conversions described in the following equation:

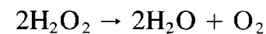


$$\text{Xanthine} + O_2 + \text{NADP}^+ + \text{ethanol} \rightarrow \text{urate} + \text{acetate} + \text{NADPH} + H^+$$

The amount of xanthine oxidized per unit time, measured by the increase in absorbance (340 nm) due to the formation of NADPH, was used as a measure of the catalytic activity of XO.[29] One unit of XO reduces 1 ηmol of NADP/min at 25°C.

SOD was assayed as described by Oberley and Spitz.[30] In this assay, xanthine-XO was used to generate a reproducible flux of O_2^-, while NBT was included as an indicator of O_2^- production. Because SOD competes with NBT for O_2^-, the percentage inhibition of NBT reduction was used as a measure of SOD. In the assay procedure, varying concentrations of tissue or blood SOD were added until maximal inhibition was obtained. One unit of activity is the amount of protein that gave half-maximal inhibition.

CAT catalyses the decomposition of H_2O_2 to give H_2O and O_2.



$$2H_2O_2 \rightarrow 2H_2O + O_2$$

In the UV range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm ($\epsilon_{240} = 0.00394$ litres mmol^{-1} mm^{-1}). The difference in absorbance (ΔA_{240}) per unit time was used as a measure of the CAT activity.[31] In calculating the activity of this enzyme, the rate constant (k) of the first order reaction was used as recommended.[31]

GSH-Px was assayed according to the method of Flohe and Gunzler.[32] In this procedure, GSSG formed as a result of GSH-Px action is continuously reduced by an excess of added GR (see below), providing a constant level of GSH. The coupled oxidation of NADPH was monitored spectrophotometrically at 340 nm to assess the activity of the peroxidase. One unit of GSH-Px oxidises 1 ηmol of NADPH/min at 25°C.

GR is a flavoprotein catalyzing the NADPH-dependent reduction of GSSG to GSH:

$$\text{NADPH} + H^+ + \text{GSSG} \rightarrow \text{NADP}^+ + 2\text{GSH}$$

The enzyme was assayed by following the oxidation of NADPH at 340 nm.[33] One unit of GR oxidises 1 ηmol of NADPH/min at 25°C.

The assay of GGT was based on the ability of the enzyme to catalyze the reaction between glucana and glycylglycine to form γ-glutamly-glycylglycine and 3-carboxy-4-nitroaniline. Because 3-carboxy-4-nitroaniline has an absorption maximum at 380 nm, and glucana does not absorb above 410 nm, catalytic activity was measured by monitoring the increase in absorption at 405 nm.[29] One unit of GGT converts 1 ηmol of glucana and glylglycine/min at 25°C.

All enzyme data, with the exception of CAT, were expressed as specific activities, that is, as units/min/mg of protein. The protein content of samples was determined by the method of Lowry et al. using standards of bovine serum albumin.[34]

The assay of total glutathione (GSH + GSSG) was based on the recycling of GSH, which is oxidized by DTNB to give GSSG with stoichiometric formation of 5-thio-2-nitrobenzoic acid (TNB). GSSG is reduced to GSH by GR and NADPH. The rate of TNB formation, which is, therefore, proportional to the sum of GSH and GSSG present, was followed at 412 nm.[35]

For determinations of vitamin C, the method of Omaye et al. was used.[36] Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products were reacted with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone. This compound, in sulphuric acid, undergoes a rearrangement to form a chromogen that is proportional to the amount of vitamin C present when measured at 520 nm.

Vitamin E was assayed according to the method of Desai.[37] In this procedure, ferric ions were reduced to ferrous ions in the presence of tocopherols, with the formation of a pink-colored complex being facilitated by adding bathophenanthroline. Absorbance of the stable chromophore, which paralleled the amount of tocopherol present, was measured at 536 nm.

Statistical analyses

The significance of results was assessed by analysis of variance after the data were logarithmically transformed to ensure homogeneity of variance.[38] All main effects and their first-order interactions were isolated and tested for significance using the within-group error mean square to calculate variance ratios. Comparisons between treatments were made using Student's *t*-test. All values presented in the tables are the means ± SEM of the nontransformed data.

RESULTS

The effects of exposing pregnant QS mice to the semiacute regime of alcohol on lipid peroxides, XO, and various antioxidant defenses were investigated in both the maternal system (liver, blood, or plasma, endometrium) and day 12-embryos. The results are presented in Table 1 and summarized in Table 3. The levels of lipid peroxides, glutathione, and vitamin E, and the activities of SOD, CAT, and GSH-Px were appreciably higher in the maternal liver than in any of the other tissues examined. Lipid peroxide levels increased significantly ($p < .05$) in the liver following exposure to alcohol, but failed to change in response to the drug in the other tissues. Additional alcohol-induced changes in the maternal liver included decreases both in the activity of GSH-Px ($p < .05$) and in the levels of glutathione ($p < .01$). Only few other alcohol-induced changes were registered in the maternal system and included decreased activity of GSH-Px ($p < .05$) in blood, decreased activity of XO ($p < .05$) and increased activity of GGT ($p < .01$) in the endometrium. Although GGT activity was assayed in all tissues, it was only detected at levels that could be measured with confidence in the endometrium. Similarly, vitamin E was not detected in blood samples using the present techniques because of the small volumes of blood available for assay.

Table 2 presents the results of a study designed to investigate the effects of exposing pregnant mice to a chronic alcohol regime on the same parameters as described above. The results are summarized in Table 3. Although the levels of most of the parameters measured in these animals were very similar to those detected in the group exposed to the semiacute regime, there were some exceptions. Notably, blood SOD activity levels in the control and treated animals in the chronic group were somewhat lower than those in the semiacute group. In contrast, glutathione levels in both the endometrium and control embryos were detected at higher levels in the chronically exposed group than in the semiacute alcohol exposed group of animals.

With respect to the changes induced by alcohol administered under the chronic regime, lipid peroxide levels in the maternal liver significantly ($p < .05$) increased, while levels of SOD ($p < .05$), GSH-Px ($p < .01$), and vitamin E ($p < .05$) significantly decreased in this tissue. Exposure to the chronic regime of alcohol also induced a significant ($p < .05$) decrease in the activity of blood GSH-Px, but caused no detectable alterations in the endometrium. In addition, XO activity and glutathione levels significantly ($p < .01$) decreased while GR activity was significantly ($p < .05$) enhanced in the embryos following chronic exposure to alcohol.

Table 1. Effects of Semiacute Administration of Ethanol on Lipid Peroxides, XO, and Antioxidant Defenses in Various Tissues of Pregnant QS Mice

	Maternal Liver		Maternal Blood or Plasma		Endometrium		Embryo	
Parameter	Controls	Alcohol Exposed	Controls	Alcohol Exposed	Controls	Alcohol Exposed	Controls	Alcohol Exposed
Lipid peroxides (ηmol/g tissue or /ml plasma)	366 ± 17 (n = 8)	437 ± 26* (n = 9)	57 ± 2 (n = 14)	63 ± 4 (n = 7)	270 ± 6 (n = 10)	289 ± 19 (n = 10)	58 ± 5 (n = 10)	67 ± 5 (n = 9)
XO (U/g protein)	2.7 ± 0.4 (n = 9)	1.9 ± 1 (n = 9)	—	—	3.6 ± 0.4 (n = 6)	2.5 ± 0.1* (n = 6)	4 ± 0.4 (n = 6)	3.8 ± 0.3 (n = 6)
SOD (U/mg protein)	470 ± 19 (n = 6)	457 ± 27 (n = 6)	63 ± 3 (n = 6)	72 ± 3 (n = 6)	59 ± 3 (n = 6)	62 ± 4 (n = 6)	51 ± 6 (n = 6)	54 ± 1 (n = 6)
CAT (k/g tissue or /ml blood)	14 ± 1 (n = 7)	13 ± 1 (n = 8)	9 ± 0.5 (n = 16)	9.3 ± 1.3 (n = 6)	1 ± 0.1 (n = 6)	0.9 ± 0.1 (n = 6)	0.7 ± 0.1 (n = 6)	0.8 ± 0.1 (n = 6)
GSH-Px (U/g protein)	482 ± 12 (n = 8)	447 ± 10* (n = 8)	139 ± 8 (n = 9)	119 ± 5* (n = 9)	38 ± 2 (n = 10)	40 ± 2 (n = 11)	26 ± 4 (n = 7)	29 ± 3 (n = 7)
GR (U/g protein)	57 ± 3 (n = 8)	54 ± 2 (n = 8)	9.6 ± 0.3 (n = 13)	9 ± 0.4 (n = 17)	48 ± 3 (n = 11)	47 ± 3 (n = 10)	29 ± 2 (n = 8)	31 ± 2 (n = 6)
GGT (U/g protein)	—	—	—	—	23 ± 1 (n = 10)	38 ± 1** (n = 6)	—	—
Glutathione (μg/g tissue or /ml blood)	3730 ± 210 (n = 8)	2550 ± 220** (n = 8)	219 ± 13 (n = 7)	210 ± 36 (n = 7)	207 ± 10 (n = 8)	201 ± 8 (n = 10)	383 ± 8 (n = 12)	349 ± 13 (n = 14)
Vitamin C (μg/g tissue or /ml blood)	394 ± 35 (n = 7)	419 ± 27 (n = 7)	27 ± 2 (n = 6)	26 ± 2 (n = 8)	408 ± 24 (n = 6)	412 ± 13 (n = 6)	274 ± 11 (n = 6)	294 ± 38 (n = 6)
Vitamin E (μg/g)	52 ± 6 (n = 6)	49 ± 7 (n = 6)	—	—	29 ± 7 (n = 6)	21 ± 4 (n = 6)	21 ± 3 (n = 6)	22 ± 2 (n = 6)

*, **, Significantly different from controls, $p < 0.05$, $p < 0.01$, respectively. n = Number of samples. Values are the means ± standard error of the mean.

DISCUSSION

A substantial literature now exists describing alcohol-induced increases in the levels of lipid peroxidation products, such as MDA.[14,17,39,40] Our findings are consistent with this phenomenon, but indicate that lipid peroxidation products increased only in the maternal liver of pregnant mice after exposure to either semiacute or chronic regimes of alcohol. The levels of lipid peroxidation products in the maternal blood, uterine endometrium, and embryos remained unchanged in response to alcohol exposure indicating that the drug failed to promote oxidative injury at these sites. Because the liver is a major site of alcohol metabolism in mammals and contains high levels of alcohol and acetaldehyde dehydrogenases, together with a variety of other metabolic systems responsible for the generation of ethanol-derived ROS and free radicals,[14,17,39,40] the hepatic effects registered in this context are not surprising. In contrast, blood, uterus, and embryo-fetal tissues contain low activity levels of alcohol dehydrogenase[41,42] and, presumably, also low levels of the other enzymatic systems that generate ROS and free radicals from ethanol. It should be noted, however, that no iron chelating compounds, such as deferoxamine, were added to any of the tissue preparations used in the assay of lipid peroxidation products because of the risk of altering the color of the malondialdehyde-TBA reaction product. Thus, while it is possible that differences in the iron content of the different tissues could account, in part, for differences in lipid peroxidation values, alcohol-induced alterations in the liver values are unlikely to be confounded with iron changes because we have recently found that these regimes of alcohol exposure fail to significantly alter iron levels in any of the tissues and organs studied (unpublished observations).

The ability of acetaldehyde, derived from the alcohol dehydrogenase-catalyzed oxidation of ethanol, to generate free radicals has been ascribed to its oxidation either by XO[14] or acetaldehyde dehydrogenase.[43] Although the affinity of XO for acetaldehyde is much less than that of acetaldehyde dehydrogenase, both enzymes are about equal in their ability to generate free radicals.[39] However, because the concentration of acetaldehyde in the liver is far below the K_m of XO, this enzyme may not be responsible to any appreciable extent for the increased alcohol-induced lipid peroxidation detected in the liver. This is supported by the finding that the activity of the enzyme failed to change significantly in the liver in response to alcohol. In contrast, the activity of this enzyme significantly decreased in both the endometrium and embryos following exposure to either the

Table 2. Effects of Chronic Administration of Ethanol on Lipid Peroxides, XO, and Antioxidant Defenses in Various Tissues of Pregnant QS Mice

Parameter	Maternal Liver		Maternal Blood or Plasma		Endometrium		Embryo	
	Controls	Alcohol Exposed	Controls	Alcohol Exposed	Controls	Alcohol Exposed	Controls	Alcohol Exposed
Lipid peroxides (ηmol/g tissue or /ml plasma)	399 ± 14 (n = 12)	452 ± 19* (n = 10)	50 ± 1 (n = 10)	51 ± 2 (n = 10)	228 ± 15 (n = 8)	282 ± 25 (n = 8)	47 ± 4 (n = 8)	61 ± 10 (n = 6)
XO (U/g protein)	2.7 ± 0.1 (n = 8)	2.5 ± 0.1 (n = 10)	—	—	3.3 ± 0.3 (n = 6)	3.9 ± 0.2 (n = 11)	3.8 ± 0.4 (n = 6)	2.1 ± 0.2** (n = 8)
SOD (U/mg protein)	463 ± 18 (n = 8)	396 ± 20* (n = 9)	47 ± 3 (n = 10)	40 ± 3 (n = 10)	49 ± 2 (n = 8)	56 ± 6 (n = 8)	49 ± 1 (n = 8)	53 ± 2 (n = 8)
CAT (k/g tissue or /ml blood)	16 ± 1 (n = 10)	15 ± 1 (n = 10)	7.5 ± 0.5 (n = 13)	6.5 ± 0.4 (n = 15)	1.1 ± 0.1 (n = 8)	1.1 ± 0.1 (n = 8)	1 ± 0.2 (n = 6)	0.7 ± 0.1 (n = 7)
GSH-Px (U/g protein)	457 ± 13 (n = 12)	284 ± 17** (n = 9)	143 ± 4 (n = 10)	127 ± 5* (n = 10)	36 ± 2 (n = 10)	38 ± 1 (n = 8)	33 ± 1 (n = 8)	32 ± 1 (n = 6)
GR (U/g protein)	67 ± 2 (n = 12)	66 ± 2 (n = 10)	8.7 ± 0.9 (n = 11)	9.4 ± 1.3 (n = 8)	54 ± 3 (n = 6)	51 ± 2 (n = 6)	36 ± 2 (n = 6)	48 ± 5 (n = 6)
GGT (U/g protein)	—	—	—	—	21 ± 4 (n = 6)	27 ± 5 (n = 10)	—	—
Glutathione (μg/g tissue or /ml blood)	3820 ± 320 (n = 8)	3830 ± 240 (n = 8)	221 ± 13 (n = 8)	257 ± 19 (n = 10)	320 ± 10 (n = 7)	308 ± 19 (n = 8)	460 ± 7 (n = 6)	385 ± 10** (n = 6)
Vitamin C (μg/g tissue or /ml blood)	391 ± 12 (n = 7)	377 ± 22 (n = 7)	19 ± 2 (n = 6)	21 ± 1 (n = 6)	382 ± 8 (n = 10)	424 ± 12 (n = 10)	276 ± 14 (n = 10)	298 ± 6 (n = 10)
Vitamin E (μg/g)	55 ± 2 (n = 7)	44 ± 4* (n = 7)	—	—	40 ± 4 (n = 6)	33 ± 3 (n = 6)	18 ± 1 (n = 6)	20 ± 2 (n = 6)

*, **, Significantly different from controls, $p < 0.05$, $p < 0.01$, respectively. n = Number of samples. Values are the means ± standard error of the mean.

semiacute or chronic regimes of alcohol, but the mechanism underlying this effect is not apparent.

Schisler and Singh[25] have previously reported that the chronic administration of alcohol to rats and various strains of mice at the same dose and under similar conditions as exploited in the present study, reduced the liver activities of total SOD, CAT, and GSH-Px in most cases. They indicated that the response of CAT to alcohol was variable between mouse strains exhibiting either repression, induction, or no change. The GSH-Px activity in the erythrocytes of young rats was also found to be reduced after chronic treatment with low doses of alcohol.[44] The significantly depressed activities of SOD and GSH-Px in the maternal liver and blood of QS mice exposed to the chronic regime of alcohol in the present study confirm these observations, and further indicate that this strain of mouse is resistant to alcohol-induced alterations in catalase activity. SOD activity did not change in any tissue studied in response to the semiacute regime of alcohol, indicating that prolonged exposure to the drug is needed in this strain of mouse to influence the activity of the enzyme. This finding is essentially at variance with that of other investigators who reported that exposure to acute doses of ethanol either decreases or increases the activity of cytosolic SOD in hepatocytes.[14,17,45]

The reported effects of long-term ethanol feeding on hepatic vitamin E are also variable[17] and depend largely on the ethanol dose administered and the vitamin E content of the diet.[14] In the present study, an alcohol-

Table 3. Summary of the Effects of Semiacute and Chronic Administration of Ethanol on Lipid Peroxides, XO, and Antioxidant Defenses in Various Tissues of Pregnant QS Mice

Treatment	Tissue			
	Liver	Blood	Endometrium	Embryo
Semiacute	lipid peroxides ↑ GSH-Px ↓ glutathione ↓	GSH-Px ↓	XO ↓ GGT ↑	No changes
Chronic	lipid peroxides ↑ SOD ↓ GSH-Px ↓ Vitamin E ↓	GSH-Px ↓	No changes	XO ↓ GR ↑ glutathione ↓

induced decline in the liver content of vitamin E was recorded only in response to the chronic administration of the drug. This finding was consistent with that of previous studies undertaken with rats.[46–48] The reduction in liver vitamin E after long-term exposure to alcohol is most likely due to its utilisation as a chain-breaking antioxidant in this tissue where it quenches the alcohol-enhanced lipid peroxy radicals resulting from increased lipid peroxidation.[17] A similar decrease in liver vitamin E content was not seen as a consequence of semiacute exposure to alcohol because the time period of increased lipid peroxidation was presumably not sufficiently long enough to deplete the vitamin through chain-breaking demands. Although the regeneration of vitamin E can be achieved by vitamin C,[17] the generation of free radicals can cause the conversion of α-tocopherol into α-tocopheryl quinone, a conversion that is irreversible under physiological conditions.[14] Because vitamin E is the main membranous chain-breaking antioxidant, its depletion by alcohol after prolonged exposure would be expected to potentiate the toxicity of the drug.[49] Supplementation of these animals with vitamin C may be useful in the regeneration of vitamin E, but because no alcohol-induced changes in the vitamin C status of any tissue were detected in the present study, its effectiveness in this capacity remains questionable.

Acute exposure to alcohol generally results in lowered hepatic glutathione levels[14,17,40] while chronic exposure induces varied results.[14] However, glutathione synthesis does not appear to be inhibited by ethanol in hepatocytes under in vitro culture conditions,[50] suggesting that ethanol-induced changes in the secretion of hormones, such as catecholamines may be involved in the liver glutathione changes reported in vivo. It has been suggested that decreased liver levels of the tripeptide occur as a consequence of increased generation of prooxidant free radicals and lipid peroxides.[51] The depressed levels of glutathione in the maternal liver after semiacute exposure to alcohol in the present study are consistent with these reports. In addition, the decreased glutathione status of the embryos following chronic exposure to the drug in the present study is also consistent with previously reported alcohol-induced glutathione depletions in the brain and liver of day-21 fetuses of rats.[52] While Reyes et al.[52] indicated that these fetal changes in glutathione were accompanied by increases in the activity of GGT, the activity levels of this enzyme in the mouse embryos in the present study were too low to measure with confidence. Because glutathione is considered to be an essential biochemical feature of the reproductive and developmental cycle, and protects the embryo from xenobiotics,[53] it may be argued that its depletion could increase the susceptibility of the embryo-fetus to the toxic effects of alcohol and the free radicals generated by its metabolism.[52] The high activity of GGT, the enzyme responsible for the degradation and recycling of glutathione via the γ-glutamyl cycle, during fetal development further suggests that the regulation of glutathione may be critical in the development of the fetus. The activity of GGT reaches a peak just before birth and then enzyme activity begins to fall toward adult levels.[54] Notwithstanding this, a depletion of glutathione by more than 90% may be required to increase susceptibility to oxidative stress effects.[55]

Differences in some of the measured parameters in the present study between the semiacute and chronic regimes may be explained by handling differences manifested as stress effects. Thus, in the semiacute studies both control and alcohol-exposed animals were handled daily for 6 d to administer ethanol or sucrose solution intragastrically, while animals treated under the chronic regime were only disturbed during the period of the experiment by general maintenance procedures.

In conclusion, the results indicate that the maternal consumption of alcohol during pregnancy in the QS mouse failed to promote lipid peroxidation in the blood, uterine endometrium, or developing embryo, and induced only minor changes in the antioxidant defenses of these tissues, even during a period of long-term chronic exposure. In contrast, alcohol increased the generation of lipid peroxides in the liver of the pregnant animals, indicating oxidative damage in this tissue, but again promoted only relatively small, though significant, changes in several antioxidant defenses that were not necessarily consistent over both the semiacute and chronic regimes of alcohol exposure. While these changes represent mechanisms that may, in part, contribute both directly and indirectly to the growth retarding and other fetotoxic effects of alcohol, it is highly unlikely that, alone, they would play a major role in this capacity in the absence of other insults generated by alcohol. Thus, because alcohol is multifactorial in its effects, the present alterations in oxidative defenses might only become important as a mechanistic component when it is totalled in the summation of events that contribute to this problem. Notwithstanding this, because the present findings stem from the QS mouse as an animal model, care should be exercised in extrapolating the results to humans when accounting for the embro-fetotoxic effects of alcohol in this species.

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ABBREVIATIONS

CAT—catalase
FAS—fetal alcohol syndrome
GGT— γ-glutamyl transpeptidase
GR—glutathione reductase
GSH—reduced glutathione
GSH-Px—glutathione peroxidase
GSSG—oxidised glutathione
MDA—malondialdehyde
QS— Quackenbush Special
ROS—reactive oxygen species
SOD—superoxide dismutase
TBA—thiobarbituric acid
XO—xanthine oxidase