± 10.59 (CFSC) nmoles min^{-1} mg^{-1} protein, and 4-HNE conjugation 9.53 ± 2.16 (NFSC) and 3.63 ± 2.53 (CFSC) nmoles min^{-1} mg^{-1} protein. Immunoblots for GST classes Ya, Yb and P1-1 demonstrate CFSC possess greater quantities of Yb and P1-1 GST; Ya was not detected. HPLC measurement of glutathione (GSH) reveled that CFSC lines displayed 3-fold higher concentrations of GSH (4.72 nmols/10^6 cells) as compared to NFSC (1.6 nmols/10^6 cells). Taken together this data suggests that CFSC have a greater capacity than NFSC to deactivate aldehydes through glutathione conjugation and oxidation to acid metabolites. (Supported by AA09300.)

951 PURIFICATION AND CHARACTERIZATION OF A MITOCHONDRIAL THYMINE GLYCOL ENDONUCLEASE FROM RAT LIVER.

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Mitochondrial (mt) DNA is exposed to damage caused by oxygen radicals produced during oxidative phosphorylation or during exposure to chemical or physical agents. Structural alterations in mtDNA and associated mt dysfunction have been correlated with aging and diseases. Accumulation of oxidative mtDNA damage may be responsible for these mtDNA alterations. mtDNA repair may therefore be important in preventing mt dysfunction. Here, we report the purification of a novel rat liver mt thymine glycol (TG) endonuclease (mtTGendo). Initially, we observed that crude protein extracts from purified rat liver mitochondria incised a radiolabeled oligonucleotide duplex containing one TG. Utilizing subsequent DEAE, cation exchange, hydrophobic interaction and size exclusion (SE) chromatography, a ~450-fold purification of the mtTGendo activity was achieved. Active SE fractions displayed a single band of ~37 kDa on a silverstained gel. mtTGendo is EDTA resistant and active within a broad KCl concentration range. Further, mtTGendo has an associated AP-lyase activity, making a similar incision into the TG-containing DNA as *E. coli* endonuclease III, the bacterial enzyme that repairs TG. Duplexed oligonucleotides containing 8-oxodeoxyguanosine or uracil and single-stranded oligonucleotide containing one TG are no substrates for mtTGendo. Given the fact that TG blocks DNA replication, this novel DNA repair enzyme may help to prevent accumulation of structural mtDNA changes, mt dysfunction and associated (patho)-physiological alterations *in vivo* that are associated with oxidative stress.

952 EFFECTS OF N-*TERT*-BUTYL-α-PHENYL NITRONE (PBN) ON DIISOPROPYLPHOSPHOROFLUORIDATE (DFP) OR KAINIC ACID (KA) INDUCED SEIZURES, FASCICULATIONS AND MUSCLE NECROSIS.

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Indirect evidence suggests that reactive oxygen species (ROS) may mediate DFP and KA induced neuronal and muscle fiber necrosis following seizures and fasciculations. Therefore the spin trapping agent PBN was used *in vivo* to assess formation of ROS during DFP and KA treatment. Pretreatment with PBN (150–300 mg/kg, i.p.) completely prevented seizures, fasciculations and necrosis induced with DFP (1.3 mg/kg, s.c.), but did not prevent seizures and fasciculations when given following DFP, nor did pretreatment with PBN prevent seizures induced by KA or pilocarpine, a muscarinic receptor agonist. DFP alone reduced AChE activity in brain to 3% and in diaphragm to 25% of control. Pretreatment with PBN attenuated this inhibition in brain to 37% and diaphragm to 80% of control. PBN given after DFP neither protected AChE from DFP inhibition nor prevented seizures and fasciculations. It is suggested that PBN has a direct, dose dependent reversible inhibitory effect on AChE of brain and muscle and thus protects the enzyme from critical irreversible inhibition by DFP. While the role of PBN as an effective antioxidant is well established its protection against DFP induced seizures, fasciculation and necrosis are not due to its antioxidant action. (Supported by NIH Grant, ES04597.)

953 ISOTOPIC DILUTION METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF 2-AMINO 6,8-HYDROXYPURINE (8ohG).

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Oxidative injury to DNA is a common cellular insult following exposures to many xenobiotic agents. The hydroxyl adduct of guanine at the C8 position (8ohG) of DNA has been associated with mutagenic base transitions and has been identified as a potential biomarker for human cancer risk. Analytic methods for identification of 8ohG include high pressure liquid chromatography (HPLC), gas chromatography mass spectroscopy (GC-MS), P^{32} postlabeling, and alkaline unwinding assays, each with inherent strengths and weaknesses. Our laboratory has evaluated the performance of an isotopic dilution method for GC-MS identification of 8ohG using a C^{13} N^{15} ring labeled internal surrogate ($8ohG_L$) with emphasis on quantifying artifactual oxidation of guanine. Data from repeat injections of the compound and replicate calibration curves over a four week period indicates that $8ohG_L$ is stable in 10mM sodium hydroxide. Precision of the method to reproducibly hydrolyze 8-hydroxyguanosine and then derivatize and quantify 8ohG was indicated by a 6.8% coefficient of variation (CV) in multiple replicate analyses of the base-sugar compound. Recovery of the 8OH-guanosine was 99%, and sensitivity was 20 pg (120 femptomoles). The detection limit (with 50 ug of DNA in a final extract volume of 100ul) was 20pg of 8ohG per ug of DNA. Replicate samples containing 50 ng of HPLC purified guanine were derivitized with bis(trimethylsilyl)-trifluoroacetatamide (BSTFA) and analyzed using this method to quantify artifactually produced 8ohG. Levels of 8ohG ranged from 500pg (1/2 detection limit) to 1200 pg, suggesting that artifactual oxidation during derivitization accounts for less than 2% of total guanine. Replicate analyses of MCF-7 carcinoma cell DNA and calf thymus DNA samples prepared by a high salt extraction procedure resulted in CVs of 22% and 23%, respectively. Ongoing work is comparing the method to HPLC using DNA samples extracted from cells and animal tissues treated with pro-oxidant agents. (Support from NIEHS #ES09455-01 and USACEHR.)

954 CORRELATION BETWEEN THE FORMATION OF 8-HYDROXY-2'-DEOXYGUANOSINE (OH8dG) AND MORPHOLOGICAL TRANSFORMATION IN SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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The formation of OH8dG is one of the most prevalent oxidative DNA modifications. Previously reported studies have suggested an association between OH8dG formation and carcinogenesis. However, it is not clear whether OH8dG formation results in the necessary genotoxic events for cancer development. In the present study, the formation of OH8dG and its ability to transform SHE cells was examined. Methylene blue, a photosensitizer which in the presence of light can generate singlet oxygen by a type II mechanism, was used to generate oxidative DNA damage. This model has been shown to predominantly produce OH8dG. Methylene blue plus light produced a dose-dependent increase of OH8dG as well as morphological transformation in SHE cells. SHE cells transfected with DNA that contained increasing concentrations of OH8dG, produced a dose-dependent increase in morphological transformation. Co-treatment of SHE cells with both β-carotene, a singlet oxygen quencher, and methylene blue and light inhibited the morphological transformation (in a dose dependent manner). These results suggest that formation of OH8dG through methylene blue and light can induce morphological transformation. In addition, these data provide further support for a role of OH8dG formation in the carcinogenesis process.

955 OXIDATIVE STRESS AND 8-HYDRODEOXYGUANOSINE IN RATS FOLLOWING ACUTE EXPOSURE TO TRICHLOROETHYLENE OR PERCHLOROETHYLENE.

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8-Hydroxydeoxyguanosine (8OHdG) is a product of DNA oxidation and has been employed in occupational studies as a marker of biologically effective dose. Trichloroethylene (TCE) and perchloroethylene (PERC) are used in the dry cleaning industry and their metabolism can produce reactive oxygen compounds with the potential to oxidize DNA. The objective of the present study was to determine if acute exposure to TCE or PERC caused oxidative DNA damage in rats that could be detected as an increase in lymphocyte

8OHdG/dG or urinary excretion of 8OHdG. Thiobarbaturic acid reactive substances (TBARS) and 8-epi -prostaglandin 2 alpha (8epiPGF2) were also measured as biomarkers of increased oxidative stress. Male Fisher rats were administered a single i.p. injection of 0, 100, 500, or 1000 mg/kg of PERC or TCE. Control rats received only vehicle (1:4 v/v of Alkamuls/water) and a positive control group received 100 mg/kg 2-nitropropane (2NP). Rats were sacrificed 24 hrs after dosing. In rats receiving 2NP or TCE, TBARS and the 8OHdG/dG ratio were significantly elevated in liver. Lymphocyte 8OHdG/dG was also elevated, but not significantly. In rats receiving 2NP, urinary excretion of 8OHdG and 8epiPGF2 were significantly increased at 12 and 24 hrs. In rats receiving TCE, no change in urinary excretion of 8epiPGF2 was evident, but an increase in 8OHdG was noted at 24 hr. Results indicate that a single high dose of TCE, can result in detectable elevation in 8OHdG. Despite an absence of statistically significant effects in rats receiving PERC, dose-dependent trends in several endpoints suggest a slight increase in oxidative stress.

956 EFFECT OF SOY ISOFLAVONE DIETARY SUPPLEMENTATION ON LEVELS OF OXIDATIVE DNA DAMAGE IN BLOOD OF WOMEN.

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Dietary intake of soy has been linked with decreased cancer risk. Some of the active compounds in soy have been identified and include the isoflavones genistein and daidzein. As part of a pilot study to assess the potential benefits of a soy extract, NovaSoy® (Archer Daniels Midland Co.), we examined levels of oxidative DNA damage in blood of six women before and after soy supplementation daily. The NovaSoy® supplement is a soy extract tablet that contains 50 mg of soy isoflavones, chiefly genistein and daidzein in a 1:1 ratio. Blood samples were obtained at weekly intervals for three weeks from the women taking this tablet once daily. DNA was isolated from the blood nuclei and analyzed for levels of 5-hydroxymethyl-2'-deoxyuridine (5-OHmdU). Levels of 5-OHmdU decreased in four of the six women. The subjects who displayed decreased levels tended to have higher baseline levels of 5-OHmdU. Average levels of 5-OHmdU were 93 $\pm$ 36 at baseline and 48 $\pm$ 15 after 3 weeks of intervention (given as mean $\pm$ SD in pg 5-OHmdU/ng thymidine, n=6 in each case). This decrease was noted as soon as 2 weeks after starting on the soy supplement, indicating a fairly rapid decrease in levels of oxidative stress. Since increased levels of 5-OHmdU have been associated with increased breast cancer risk, these results indicate that soy supplementation could potentially be useful to decrease breast cancer risk in women.

957 OXIDATION OF LOW DENSITY LIPOPROTEIN BY HYDROGEN PEROXIDE-ALTERED MYOGLOBIN.

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Oxidized low density lipoprotein (Ox-LDL) is believed to play a significant role in the pathogenesis of atherosclerosis; however, the exact mechanism by which Ox-LDL is formed *in vivo* is not yet known. A variety of biological molecules, including the hemoproteins myoglobin and hemoglobin, have been shown to induce the formation of Ox-LDL *in vitro*. We have discovered, in *in vitro* studies with purified LDL, that hydrogen peroxide-altered myoglobin was approximately 2.5-fold more efficient than native myoglobin (Mb) at oxidation of LDL, as demonstrated by the formation of lipid peroxides, TBARS, and conjugated dienes. The oxidation of LDL appeared to be catalyzed by a peroxide-altered heme group covalently-bound to apomyoglobin (Mb-H). We have also discovered that Mb-H was formed during the reaction of Mb with purified LDL. Therefore, the previously observed capacity of Mb to oxidize LDL may be due, in part, to the generation of Mb-H. These results are consistent with the known redox activity of Mb-H. The formation of covalently-bound heme adducts has been shown to occur *in vivo* with a variety of hemoproteins. Thus, these observations suggest a potential role for oxidatively-modified hemoproteins in the initiation and progression of atherosclerosis. (Supported by BWF New Investigator Award in Toxicology (YO), Training Grant GM07767 (JLV), and ES08365.)

958 H_2O_2 INDUCES HYPERTROPHY AND APOPTOSIS IN RAT CARDIOMYOCYTES.

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Cardiovascular disease is the leading cause of nature death in U.S. Many heart diseases involve cardiomyocyte hypertrophy and cell death. The trigger and mechanism of these two cellular changes are unknown under most pathological conditions. Oxidants are by-products of aerobic metabolism and increase in the heart in many heart diseases associated with ischemia-reperfusion. Using H9C2 and primary cultured rat cardiomyocytes, we test whether H_2O_2 can induce hypertrophy and apoptosis. The majority of cells survived a 2-hr treatment with H_2O_2 up to 250 μM. These cells developed hypertrophy over 6 days judging by increases in cell surface area, protein content per cell and protein to DNA ratio. The enlarged cells showed enhanced actin filaments. Myosin was presented in a filament structure instead of myofibrils. Protein degradation rate showed a transient reduction but increased at 24 hr after H_2O_2 treatment, suggesting that increased protein content was not a result of reduced protein degradation. Cycloheximide prevented cell enlarging, indicating that new protein synthesis was critical for hypertrophy. In contrast, the same treatment, i.e. H_2O_2 =250 μM, 2 hr, caused a maximum of 45% cells to undergo apoptosis over 24 hr. These cells detached, showed nuclear condensation, and activated caspase-3. Thus, our data indicate that H_2O_2 can cause apoptosis in one fraction of the cardiomyocytes and hypertrophy in the other fraction.

959 CARDIOSELECTIVE OXIDATION OF MITOCHONDRIAL DNA FOLLOWING SUBCHRONIC ADMINISTRATION OF DOXORUBICIN.

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Acute intoxication of rats with doxorubicin causes the preferential formation of 8-hydroxydeoxyguanosine (8OHdG) in mitochondrial DNA (mtDNA) compared to nuclear DNA (nDNA) of cardiac tissue (Palmeira et al., 1997). The present investigation was designed to assess the persistence of these adducts and to determine whether they accumulate with repetitive injections of clinically relevant doses of doxorubicin. Adult male Sprague-Dawley rats received weekly intraperitoneal injections of either 2 mg/kg doxorubicin or isotonic NaCl. Selected animals were killed at weekly intervals, the nDNA and mtDNA isolated from both heart and liver, and the concentration of 8OHdG measured by LC/ESI-MS/MS. The concentration of 8OHdG in nDNA and mtDNA from heart or liver of saline-treated rats was less than 5 per 100,000 unmodified deoxyguanosine (dG) bases at all time points examined. However, the abundance increased to 65/10^5 dG in cardiac mtDNA after 8 weekly injections. The rank-order abundance of 8OHdG adducts was cardiac mtDNA > hepatic mtDNA > cardiac nDNA > hepatic nDNA. Interestingly, the concentration of 8OHdG did not decline in any of the DNA fractions after allowing as much as 3 weeks of drug-free recovery. This preferential oxidation of cardiac mtDNA is consistent with the bioenergetic failure and the cumulative and irreversible cardiomyopathy that limits the clinical utility of this important antineoplastic drug. (Supported in part by HL 58016.)

960 PYRIDOSTIGMINE AND EXERCISE INTERACTION ON CARDIAC ANTIOXIDANT DEFENSE SYSTEM IN MICE.

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This study investigated the interactive effects of pyridostigmine and exercise on cardiac antioxidant defense system in mice. Male NIH Swiss mice were divided into four groups and treated as follows: 1) sedentary control (4 weeks sitting on treadmill belt followed by two weeks saline treatment and 4 weeks sitting on treadmill belt); 2) pyridostigmine [4 weeks sitting on treadmill belt followed by pyridostigmine (1.2 mg/kg, p.o) daily for two weeks and 4 weeks sitting on treadmill belt]; 3) exercise daily for ten weeks (saline treatment only at 5th and 6th week) and 4) pyridostigmine plus exercise for ten weeks. The animals were sacrificed 24 hours after the last treatments, hearts were isolated and analyzed. Pyridostigmine significantly increased cardiac SOD activity (125% of control) while GSH-Px activity

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 38th Annual Meeting of the Society of Toxicology, held at the Ernest N. Morial Convention Center, New Orleans, Louisiana, March 14-18, 1999.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 419.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 444.

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