chromium-induced carcinogenesis which involves the persistent stimulation of growth regulatory pathways. (Supported by DE&RF (#B11/I-93) & NJCCR (#690-043) to E.J.Y.).

1668 EFFECTS OF ANTIOXIDANTS ON CHROMIUM(VI)-INDUCED ROS PRODUCTION IN A549 HUMAN LUNG CELLS

H S Park, E Yurkow, S O'Connell [1], C M Witmer. *Joint Graduate program in Toxicology, EOHSI, Rutgers University, Piscataway, NJ; [1] Heartland Biotechnologies, Davenport, Iowa*

The production of reactive oxygen species (ROS) during the intracellular reduction of Cr(VI) was studied in A549 cells using flow cytometry analysis. Cr(VI) produced dose-related ROS with addition of $K_2Cr_2O_7$ (20 and 100 uM) in A549 cells. Cr(III) as $CrCl_3$ (200 uM) caused no increase in ROS. Chromium picolinate (200 uM) caused fewer ROS than controls. Cells preincubated with 200 uM vitamin C (C) (20 hr) followed by $K_2Cr_2O_7$ (100 uM) treatment produced fewer ROS than $K_2Cr_2O_7$-treated cells. Vitamin E (20 uM) preincubation (20 hr) caused no changes in ROS with $K_2Cr_2O_7$ (100 uM). However, there is increased production of ROS when C is added only 60 min prior to the Cr(VI) addition to the cells. Catalase (1000 U/ml) and/or superoxide dismutase (100 U/ml) preincubation (90 min) did not cause any change in ROS production caused by $K_2Cr_2O_7$ (100 uM) treatment. $K_2Cr_2O_7$ treatment (1, 5, 10 and 20 uM) for 24 hr caused S-phase cell cycle specific changes in DNA. The effect was maximal at 10 uM.

1669 SEX AND ORGAN SPECIFIC EFFECTS OF HEXAVALENT CHROMIUM (Cr(VI)) ON CYTOCHROME P450 (P450) ISOZYMES

E C Faria, N Sadrieh [1], P E Thomas, C M Witmer. *Joint Graduate Program in Toxicology, Rutgers University/UMDNJ-RWJMS, Piscataway, NJ; [1] NIH, National Cancer Institute, Bethesda, Maryland*

Recent studies have shown that Cr(VI), a putative carcinogen, affects the mixed function oxidase system. These studies characterize the effects of Cr(VI) on hepatic and pulmonary P450 isozymes from male and female Sprague-Dawley rats. Microsomal fractions were isolated from rats after IP injection with 130 μmoles Cr(VI)/kg/48 hours as $K_2Cr_2O_7$ (pH 7.4) for 12 days. Control animals received saline. P450 isozyme catalytic activities were determined using the *in vitro* metabolism of testosterone to its hydroxylated metabolites. The levels of P450 isozymes were measured by Western blot analysis using monoclonal antibodies (values = % of control). In male rats, Cr(VI) induced pulmonary CYP2B1 (191 ± 9%), whereas, in the liver it decreased CYP2C11 (31 ± 4%) and CYP3A2 (60 ± 9%) levels. In female rats, induction was seen in pulmonary CYP2B1 (179 ± 4%), as well as hepatic CYP2B1 (180 ± 13%) and CYP3A2 (351 ± 20%). In all tissues examined, the changes in isozyme levels were correlated with similar changes in catalytic activities. (NIEHS Training Grant ES07148, ECF; R01-GM44982, PET).

1670 DIFFERENTIAL EFFECTS OF GOLD THIOMALATE IN LIVERS AND KIDNEYS OF RATS AND MICE

E Cheriathundam, A P Alvares. *Dept. of Pharmacology, Uniformed Services University, School of Medicine, Bethesda, MD*

Gold thiomalate (AuTM) is used chronically in the treatment of rheumatoid arthritis with variable response between individuals and is often associated with transient proteinuria. In these studies, the effect of AuTM on livers and kidneys of rats and mice were examined. Benzo(a)pyrene hydroxylase activity was significantly decreased in kidneys of AuTM (100 mg/kg)-treated rats whereas no significant change was observed in the kidneys of Swiss-Webster mice treated with the same dose of AuTM. Blood urea nitrogen, an indicator of kidney dysfunction, was significantly increased in AuTM-treated rats whereas no significant difference was observed in AuTM-treated mice. Liver benzo(a)pyrene hydroxylase activity was not significantly altered in rats or mice treated with AuTM. Both liver and kidney metallothioneins were induced significantly in AuTM-treated rats and mice. Since susceptibility to metal toxicity differs among inbred strains of mice, two strains of mice were examined for AuTM toxicity. C3H/HeJ and DBA/2J inbred strains have been shown previously to be sensitive and resistant to cadmium-induced hepatotoxicity, respectively. However, AuTM did not cause any significant change in the benzo(a)pyrene hydroxylase activity in the kidneys of either strains of mice. These data indicate AuTM, a nephrotoxic agent in rats, showed no nephrotoxic effects in the mice strains studied here.

1671 ROLE OF MONOCYTE-DERIVED CYTOKINES IN THE INDUCTION OF α1-ACID GLYCOPROTEIN-2 GENE EXPRESSION BY HEAVY METALS

Mark Merchant, John Papaconstantinou. *Dept. of Human Biol. Chem. and Genetics, The Univ. of Texas Medical Branch, Galveston, TX*

α1-Acid glycoprotein-2 (AGP-2) is a liver specific, acute-phase gene which is highly inducible by bacterial lippopolysaccharide (LPS) and heavy metals. The induction of AGP-2 by LPS involves the stimulation of monocytes to produce cytokines (primarily IL-6 and TNFα) which act at the liver to induce transcription of the AGP-2 gene. This research was conducted to investigate the possibility that heavy metals induce AGP-2 gene expression via a similar mechanism. Treatment of cultured human monocytes with 20 μg/ml LPS resulted in the secretion of IL-6 (2313 ± 20 pg/ml) and TNFα (482 ± 41 pg/ml) into the media. However, treatment of monocytes with 1 μM $HgCl_2$, $CdCl_2$, or $ZnCl_2$ resulted in no statistical change in secreted IL-6 or TNFα. Human hepatoma (Hep G2) cells were transfected with a plasmid, p(−532)AGP2LUC, containing the firefly luciferase gene under the control of the AGP-2 proximal promoter. Treatment of these Hep G2 cells with media from $HgCl_2$-, $CdCl_2$-, or $ZnCl_2$-conditioned monocyte cultures resulted in no transient luciferase activity. In contrast, treatment of the transfected Hep G2 cells with LPS-conditioned monocyte media produced a 23-fold induction of luciferase activity. In addition, injection of Balb/c mice intraperitoneally with 50 μg/kg LPS resulted in a 33- and 78-fold induction of serum IL-6 and TNFα levels, respectively. However, injection of 0.5 mg/kg $HgCl_2$, $CdCl_2$, or $ZnCl_2$ resulted in no statistical change in circulating IL-6 or TNFα levels. Results from these experiments indicate that the induction of AGP-2 gene expression by heavy metals is not mediated by monocyte-derived cytokines. (Supported by DHHS-5T32ES07254-04 and the Shriners Burn Institute, Galveston, TX).

1672 DIETARY IRON LOADING GENERATES HYDROXYL RADICAL IN RATS: AN ESR SPIN TRAPPING INVESTIGATION

M B Kadiiska, Q-H Xiang, R P Mason. *Laboratory of Molecular Biophysics, National Institutes of Health, National Institute of Environmental Health Sciences, Research Triangle Park, NC*

Although iron is an essential nutrient, the pathological processes associated with various forms of iron overload demonstrate that the metal can also be toxic. We have already provided ESR evidence for the generation of hydroxyl radical during acute iron poisoning. In this study we investigated the possibility of hydroxyl radical generation by chronic dietary iron loading. A secondary-radical spin-trapping technique was employed where hydroxyl radical forms methyl radical upon reaction with DMSO. The methyl radical adduct was then detected by ESR spectroscopy as its adduct with the spin trap phenyl-*N*-*t*-butylnitrone (PBN). This adduct was detected in the bile of rats 10 weeks after being on an iron-loading diet and 40 minutes after the injection of the spin trap PBN dissolved in DMSO. Bile samples were collected into solution of the ferrous stabilizing chelator dipyridyl in order to prevent the generation of radical adducts *ex vivo* during bile collection. The analysis of radical adducts excreted *via* the biliary route provides ESR evidence for the generation of the hydroxyl radicals during chronic iron-loading intake. No radical adducts were detected in rats fed the control diet for the same period of time. This is the first report of hydroxyl radical generation detected by ESR in by chronic iron-loaded rats.

1673 THE ROLE OF FERROUS IRON IN DUST-INDUCED CARCINOGENESIS

X Huang [1], H Pezerat [2], Z Elias [3], M Costa [1]. [1] *Nelson Institute of Environmental Medicine, NYU Medical Center, Long Meadow Road, Tuxedo, NY; [2] Laboratoire de Reactivite de Surface et Structure, Universite P et M Curie, Paris, France; [3] Institut National de Recherche et de Securite, Vandoeuvre, France*

Increasing evidence demonstrates that an excess of lung cancer and stomach cancer has been observed in iron ore and steel factories workers. It has been shown that the incidence of cancer is related to the exposure to mineral dusts. Moreover, many studies have shown that the iron content of the asbestos fibers has an important role in the generation of reactive oxygen species (ROS) and in the induction of mesothelioma. In the present study, we found that $FeSO_4$ in aqueous solution and aged pyrite (FeS_2) which contained a coating of $FeSO_4$ produced ROS following interaction with O_2, as detected by the spin trapping agent DMPO with ESR. We have also found that $FeSO_4$ in solution is toxic to Syrian hamster embryo (SHE) cells, but not transforming. Aged pyrite with a coating of $FeSO_4$ was not very toxic, but transformed SHE cells. These results

suggest that very little Fe^{2+} ions can get into cells and ROS resulting from interaction of water soluble $FeSO_4$ and O_2 may damage cell membrane which causes cell toxicity. The aged pyrite which was phagocytized and subsequently released Fe^{2+} in SHE cells induced transformation of cells. Therefore, the role of reactive Fe^{2+} in airborn dusts is a parameter which is worth considering in order to predict which dusts will lead to a higher incidence of cancer. The mechanism of Fe^{2+}-containing dust-induced cell transformation is being studied.

1674 MEASUREMENT OF URANIUM IN BONE USING AN X-RAY FLUORESCENCE TECHNIQUE

I M Shapiro, P Bloch. *University of Pennsylvania, Schools of Dental Medicine and Medicine, Philadelphia, PA*

Transuranic elements such as those present in depleted Uranium (U) can pose a serious health hazard. Current approaches to the measurement of U include evaluation of its concentration in urine and whole body counting. Since U is accumulated in bone and there are both theoretical and practical problems associated with measurement of daughter products there is need for more accurate assessments of U exposure. The objective of this study was to measure U by an X-ray fluorescence (XRF) technique *in situ*. We excited the K shell electrons in U which have a binding energy of 115.6 KeV with 122 and 136 KeV gamma rays from a Co-57 source. A liquid nitrogen cooled intrinsic Ge detector was employed to measure the characteristic K fluorescence from the U as well as coherently scattered gamma rays. The quantity of U-238 in the bone was determined from the number of K fluorescence events extracted from the measured scattered photon spectrum. The bone mineral content was estimated by measuring the number of coherently scattered gamma rays. In this way we were able to relate the U concentration to the bone mass. Using this procedure it was possible to measure micromolar concentrations of U with high precision and reproducibility. This new technique overcomes limitations that are commonly seen with both urine analysis and whole body counting.

1675 TISSUE REPAIR AND INJURY AS A RESPONSE TO THIOACETAMIDE HEPATOTOXICITY: A NOVEL DOSE-RESPONSE RELATIONSHIP

R S Mangipudy, S Chanda, H M Mehendale. *Division of Pharmacology and Toxicology, College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, LA*

Stimulated cell division and tissue repair are known to a play a pivotal role in the final outcome of toxicity. The objective of this study was to measure tissue repair and injury as simultaneous but opposing biological responses to a model hepatotoxicant in a dose-response paradigm. Male S-D rats were treated with a 12-fold dose-range of thioacetamide (TA, 50, 150, 300 and 600 mg/kg). Serum ALT elevation and liver histopathology were used as markers of injury while ^{3}H-T incorporation and proliferating cell nuclear antigen (PCNA) study were used as markers of cell division and tissue repair. All responses were measured over a time course of 0–96 hr. Significant ALT elevation was noted after 48 hr only in the group receiving 600 mg/kg. Tissue repair indicated by ^{3}H-T incorporation peaked at 36 hr after administration of a low dose of TA (50 mg/kg). With increasing doses a greater but delayed stimulation of cell division was observed till a threshold was reached (300 mg/kg). Above this threshold (600 mg/kg), tissue repair was both delayed and attenuated leading to animal death. Therefore, timely and adequate tissue repair response restrained the progression of injury in a 6-fold dose-range (50, 150, and 300 mg/kg) while too little and too late repair response led to an unrestrained progression of injury in the group receiving 600 mg/kg. If tissue repair is a critical determinant of the final outcome, then intervention with this process should result in lethality even from non-lethal doses. Antimitotic administration of colchicine (CLC) to inhibit cell division in the 150 and 300 mg/kg treated groups led to 60% and 100% lethality, respectively. Thus, intervention with the repair response resulted in lethality with ordinarily non-lethal doses. These findings underscore the pivotal role of tissue repair as the determinant of the ultimate outcome of liver toxicity. Measuring both tissue repair and injury might increase the usefulness of dose-response paradigms in predictive toxicology. (Supported by The Burroughs Wellcome Fund).

1676 STRAIN DIFFERENCES IN TISSUE REPAIR RESPONSE TO A TOXIC INSULT BY 1,2-DICHLOROBENZENE

S G Kulkarni, H M Mehendale. *Division of Pharmacology and Toxicology, College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, LA*

Fischer 344 (F344) rats are reportedly 75-fold more sensitive than Sprague Dawley rats (S-D) to 1,2-dichlorobenzene (o-DCB) hepatotoxicity. However, no information is available regarding the ultimate consequence of this sensitivity on animal survival. Lethality studies were performed in the 2 strains. LD50 values in male rats for o-DCB were 1.66 ml/kg (1.18–2.355 ml/kg: 95% C.I.) in F344 and 1.76 ml/kg (0.856–3.652 ml/kg: 95% C.I.) in S-D rats. Previous studies have shown the importance of tissue repair which occurs as an endogenous response to injury leading to animal survival. Therefore, the objective of this study was to investigate if increased cell division and tissue repair underlie the observed recovery of F344 rats despite higher liver injury. Age-matched male S-D rats (250–300 g; 8/9 weeks old) and male F344 rats (160–190 g; 8/9 weeks old) were used in the study. A dose response study was performed in which injury and tissue repair occurring as two dynamic but opposing events were measured over a time course (0–96 hr). Liver injury was studied by measuring plasma ALT and SDH levels and liver function was evaluated by measuring plasma bilirubin levels. Higher plasma ALT elevations were observed in F344 rats around 36–48 hr. Plasma glucose and hepatic glycogen were used as a measure of energy status of the liver. Greater depletion of hepatic glycogen was seen in F344 rats which returned to normal by 96 hr. Liver regeneration was observed by measuring ^{3}H-thymidine incorporation into hepatonuclear DNA. Increased incorporation of ^{3}H-thymidine was seen in the F344 rats around 36–48 hr signifying an increase in S-phase synthesis. This differential in tissue repair may play a vital role in equalizing the ultimate outcome of toxicity in the two strains of rats. (Supported by the Burroughs Wellcome Fund).

1677 RETINOL PROTECTION OF ACUTE CADMIUM CHLORIDE-INDUCED HEPATOTOXICITY IN THE MALE SPRAGUE-DAWLEY RAT

A Baines, J-M Sauer, S B Hooser, I G Sipes. *Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ*

Cadmium is a toxic heavy metal which is an environmental contaminant found in meats, fishes, and fruits. The major toxicologic effects of cadmium are to the pulmonary and renal systems during chronic exposure, but when injected parenterally, it can cause acute hepatic, testicular, and cardiac injuries. In the liver, cadmium causes primarily damage to the parenchymal hepatocytes. Recently, pretreatment with retinol for 1 or 7 days has been shown to potentiate the hepatotoxicity of carbon tetrachloride in rats. Therefore, the objectives of these experiments were to evaluate the ability of retinol to modulate the liver toxicity associated with intravenous administration of cadmium chloride ($CdCl_2$) using both a 1 and 7 day retinol pretreatment regimen. Male Sprague-Dawley rats were pretreated with *all-trans*-retinol (75 mg/kg/d; po) for 1 or 7 days. Twenty-four hr after the last dose of retinol, $CdCl_2$ (2.5, 3.0, or 4.0 mg/kg; iv) was administered. Rats pretreated with retinol vehicle and given $CdCl_2$ exhibited liver injury as measured by increased plasma ALT activity and liver histology at 24 hr after receiving $CdCl_2$. Histologically, there was mild, diffuse hepatocellular degeneration and necrosis. Retinol pretreatment for 1 day had no effect on $CdCl_2$-induced hepatotoxicity. However, seven days of retinol pretreatment resulted in protection from $CdCl_2$-induced liver damage, significantly decreasing plasma ALT activity and associated liver histopathology. The mechanism(s) by which retinol protects the liver from $CdCl_2$ is unknown. However, current studies are focusing on retinol-induced increases in protective proteins. (Supported in part by NIEHS Center Grant, P30 ES 06694 and ES 06095).

1678 KUPFFER CELL ACTIVATION AFTER TRAUMA

T Huynh, L Bracey, C C Baker, J J Lemasters. *Depts. of Surgery and Cell Biology, University of North Carolina, Chapel Hill, NC*

Kupffer cells (KC) may play an important role in the immune response after injury. This study tested the following hypotheses: 1) femur fracture (FF) activates KC leading to increased hepatic O_2 uptake, 2) KC activation leads to altered responses to lipopolysaccharide (LPS), and 3) KC depletion reduces mortality associated with FF and cecal ligation and puncture (CLP).

Methods: Under Ketamine and Xylazine anesthesia, Sprague-Dawley rats were subjected to closed FF with and without $GdCl_3$ (Gd, 7 mg/kg) pretreatment to deplete KC. Subsequently, livers were perfused for measurement of O_2 uptake or for KC isolation. Isolated KC were stimulated with LPS, and nitric oxide (NO) formation was measured. In other experiments, CBA/J mice were subjected to FF and/or CLP 72h later, with or without Gd pretreatment.

Results: Hepatic O_2 uptake increased 17% and 19%, respectively, 2 h and 48 h after FF. Gd pretreatment reduced O_2 uptake to levels in sham-operated controls. FF also caused a 59% decrease in KC NO formation. In mice, CLP caused 44% mortality, which increased to 60% after CLP plus FF. Gd reduced mortality to 13% and 5%, respectively, after CLP and CLP plus FF.

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 34th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 5-9, 1995.

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The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 351.

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