1657 DMPS-MERCURY CHALLENGE TEST: URINARY MERCURY AFTER EXPOSURE TO MERCUROUS CHLORIDE

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The DIMAVAL (DMPS) challenge test (300 mg, po after an 11 hr fast) was given to 8 users of a skin lotion that contains mercurous chloride, 11 factory workers who make the skin lotion and 9 controls who had no known occupational exposure to Hg in Tampico, Mexico. Urines were analyzed for total Hg using cold vapor AAS. The μg Hg excreted for 6 hrs before and 6 hrs after DMPS treatment was 16.2 ± 3.43 and 1410 ± 346 for skin lotion users (n = 8); 113 ± 26 and 5037 ± 682 for factory workers (n = 11); and 1.08 ± 0.61 and 28.6 ± 12.1 SE for controls (n = 9), respectively. The increases in urinary Hg resulting from the DMPS challenge test were 87, 45 and 26-fold, respectively. Urinary coproporphyrin, precoproporphyrin and pentacarboxylporphyrin levels in all 3 groups decreased after DMPS administration. Coproporphyrin concentration before DMPS challenge was strongly correlated with urinary Hg excretion after DMPS challenge for the factory workers (r = 0.73) and the lotion users (r = 0.83). The results show the value of DMPS in humans for increasing urinary excretion of Hg and the value of the DMPS/MERCURY challenge test and urinary porphyrins for determining mobilizable Hg. (Supported in part by the Superfund Basic Research Program NIEHS Grant #ES-04940.)

1658 HUMAN AND RABBIT RENAL CORTICAL SLICES IN AERATED SUBMERSION CULTURE AS A MODEL TO EVALUATE HEAVY METAL CHELATORS

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Heavy metal chelators have been used for many years as therapeutic agents against metal poisonings by mercury, lead and arsenic. A submersion culture system has been developed to evaluate the efficacy of heavy metal chelators in removing metals from renal tissue. Slices were exposed to non-toxic loading doses of $HgCl_2$ (10 uM) and $NaAsO_2$ (50 uM) for 4 hrs prior to being transferred to the media (DME/F-12) containing the chelators 2,3-dimercapto-1-propane sulfonic acid (DMPS) or meso-dimercaptosuccinic acid (DMSA). DMPS and DMSA (0.5–10 mM) removed $NaAsO_2$ and $HgCl_2$ in a dose-dependent manner. Over 80% of the metals could be removed by 10 mM DMPS in 4 hrs of incubation. DMPS routinely removed more metal than DMSA. Even the lowest concentration of chelator (0.5 mM DMPS) removed 60% of the $HgCl_2$ from the renal slice, as compared to slices not exposed to chelators. The chelators were equally effective with either human or rabbit renal slices. The results of these experiments indicate that human and rabbit renal cortical slices are efficient models to evaluate heavy metal chelators as potential therapeutic agents. (NIEHS P42-ES-04940 and RO1-ES-05790).

1659 CHELATING AGENTS IN THE TREATMENT OF EXPERIMENTAL MANGANESE INTOXICATION

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The effects of seven structurally different metal chelators in the treatment of semichronic manganese (Mn) intoxication were assessed in male Swiss mice. Groups of 15 animals received daily s.c. injections of Mn (II) chloride tetrahydrate (32 mg/kg) for four weeks. Forty-eight hr after the last injection, chelation therapy was initiated and continued for 5 days. Mice received daily i.p. injections of 0.9% saline (control group), or cyclohexanediaminetetraacetic acid (CDTA), ethylenglycol-bis-(alfa-aminoethylether)-N,N'-tetraacetic acid (EGTA), N-(2-hydroxyethyl) ethylendiamine triacetic acid (HEDTA), isonicotinic acid hydrazine (INH), L-dopa, sodium 4,5-dihydroxy-1,3-benzenedisulfonate (Tiron) and p-aminosalicylic acid (PAS) at doses approximately equal to 1/8 of their respective LD_{50} values. Animals were housed in metabolic cages, and urine and feces were daily collected for 5 days. After this period, the animals were killed and tissues removed. Although none of the chelators increased the fecal Mn excretion, CDTA, EGTA, and HEDTA significantly enhanced the excretion of Mn into urine. Tiron, INH, and EGTA had no beneficial effect on tissue Mn concentrations, whereas PAS, HEDTA, and L-Dopa were only partially effective in reducing the tissue levels of Mn. In contrast, CDTA caused a significant decrease in the concentration of Mn in bone, brain, kidney and liver, the main target tissues of Mn accumulation. CDTA was the most effective chelator of those studied in the removal of Mn in Mn-loaded mice.

1660 RESPONSES OF LACTATING COWS TO MESO-2-3-DI-MERCAPTO SUCCINIC ACID (DMSA) USED TO INCREASE CLEARANCE OF HEAVY METALS

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DMSA has shown promise as a chelator to reduce burdens of metals in humans. Neither efficacy nor safety of this therapeutic agent has been demonstrated for cattle. Four lactating Jersey cows (521 ± 12 kg) were given 2 iv doses at 24 hr intervals of 0, 5, 15, or 45 mg/kg BW of DMSA in 400 ml of 5% $NaHCO^3$. Blood, urine, and milk were collected to determine changes in heavy metals. Unanticipated clinical signs of toxicosis, anorexia and depression, were evident within 24 hr of the first 15 and 45 mg/kg DMSA doses. For the 0, 5, 15, and 45 mg/kg treatments, urine production decreased by 22, 47, 74, and 85%, respectively, during the second 24 hr. By 72 hr, daily milk production decreased 18, 45, 100, and 100% from the mean of the 7 day predose period. Peak blood creatinine/BUN (mg/dl) was 0.8/9.0, 1.0/11.0, 15.7/192.0, and 10.2/127.0, respectively. The 15 mg/kg treated animal was moribund 9 days after the first dose. Necropsy revealed severe acute renal tubular necrosis with protein and cellular casts. The cow dosed with 45 mg/kg was recovering by day 15, with blood creatinine and BUN approaching normal and limited production of milk returning. Kidneys of cattle appear to be a target organ for DMSA and the threshold of toxicity should be determined.

1661 ROLE OF CALCIUM AND PHOSPHOLIPASE IN POTASSIUM ANTIMONYL TARTRATE-INDUCED CARDIAC MYOCYTE TOXICITY

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Exposure of rat cardiac myocytes to potassium antimonyl tartrate (PAT) has been shown to cause oxidative stress and toxicity. This study investigates the roles of intracellular calcium ($Ca^{2+}i$) and phospholipase (PLPase) activation in PAT-induced toxicity. Myocytes isolated from rat neonates were cultured on plastic culture dishes or glass coverslips and were used after 2–3 days in culture. Toxicity and lipid peroxidation were assessed by release of lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS), respectively. $Ca^{2+}i$ was monitored on an inverted phase contrast microscope with the fluorescent probe fura-2. ATP content was measured by extraction and HPLC analysis. Exposure to 200 μM PAT for 4 hrs resulted in a 50–60% release of LDH (% of total) and a 10-fold increase in TBARS release. In individual fura-2 loaded myocytes, 200 μM PAT increased $Ca^{2+}i$ by approximately 75% by 3 hours, whereas, exposure to 500 μM PAT resulted in $Ca^{2+}i$ overload (greater than 10-fold increase). Preloading of myocytes with the Ca^{2+} chelator BAPTA (10 μM) prevented the effects of PAT on LDH and TBARS release, but PAT exposure in calcium-free/EGTA medium did not prevent toxicity, suggesting release from intracellular Ca^{2+} stores. Co-exposure to the PLPase inhibitor mepacrine also prevented PAT toxicity. After 6 hrs exposure to PAT (200 μM) in the presence of mepacrine (50 μM) LDH release was still prevented, but myocytes were hypercontracted, suggesting an elevated $Ca^{2+}i$. PAT-induced ATP loss was partially prevented by BAPTA and mepacrine. These results link elevated $Ca^{2+}i$ and activation of PLPase with PAT-induced myocyte cell death and suggest that increased $Ca^{2+}i$ precedes phospholipase activation.

1662 EFFECTS OF ANTIMONY ON MITOCHONDRIAL FUNCTION AND PROTEIN THIOL AND ADENINE NUCLEOTIDE STATUS IN CULTURED CARDIAC MYOCYTES

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Antimony and antimony containing compounds are used in the manufacture of paints, pigments, ceramics, pyrotechnics, fire retardants and glass. Exposure to antimony containing compounds has been associated with cardiac toxicity in man and experimental animals. In a previous study in our laboratory, we demonstrated that potassium antimonyl tartrate (PAT) depletes cellular glutathione and induces oxidative stress and toxicity in rat cardiac myocytes. In the present study, cardiac myocytes isolated from neonatal rats and cultured for two days were used to further examine the mechanism of PAT-induced

toxicity. Exposure to 100 μM PAT for 4 hrs reduced the mitochondrial membrane potential by about 30% as measured by rhodamine 123 retention. The effects of PAT on cellular ATP levels were assessed by measuring total adenine nucleotides by HPLC with uv detection. PAT produced a concentration-dependent reduction in ATP levels. Concentrations of 50 and 100 μM PAT reduced cellular ATP levels by 20% and 70% respectively after 4 hrs. However, ATP was not depleted after a 2 hr exposure to these concentrations of PAT. PAT-induced losses of ATP could not be accounted for by reciprocal increases in either ADP or AMP concentrations. The effects of PAT on protein thiol levels were examined by reacting cellular proteins with the fluorescent thiol labeling probe monobromobimane. At a concentration of 100 μM, PAT produced a 40% reduction in protein thiols after 4 hrs. A significant reduction (15%) in protein thiols was also seen following a 4 hr exposure to 50 μM PAT. These results suggest that PAT has deleterious effects on mitochondrial integrity, cellular thiol status and the energy status of the cell.

1663 EFFECTS OF ALUMINUM INGESTION ON BEHAVIOR IN YOUNG, ADULT, AND OLD RATS

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Both aluminum (Al) and aging have been associated with neurobehavioral changes in mammals. In the present study, the effects of prolonged Al exposure on behavior were assessed in young (21 day old), adult (8 months), and old (16 months) male Sprague-Dawley rats. Each age group received Al nitrate nonahydrate in drinking water at doses of 0, 50, or 100 mg Al/kg/day for 6.5 months. During that period, citric acid (0, 355, or 710 mg/kg/day) was also added to drinking water. Solution concentrations were prepared daily to adjust the dose to achieve a constant intake by taking into account the differences in body weight and fluid intake. Behavioral testing consisted of assessments of horizontal and vertical activity in an open-field (OP) and passive-avoidance conditioning (PA). There were no Al effects on OF behavior in any age group. Also, there were no significant differences among dose groups in PA test latencies in the young rats. Irrespective of Al treatment, adult and old animals showed low PA conditioning. In conclusion, Al ingestion at levels of 50 and 100 mg/kg/day would not influence neurobehavioral function as indexed by OF activity in young, adult or old rats and by PA conditioning in young rats.

1664 DIFFERENTIAL TOXICITY OF TRIMETHYLTIN IN FOUR INBRED STRAINS OF MICE

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Trimethyltin (TMT) is a relatively selective neurotoxin that affects primarily the granule cells of the hippocampal fascia dentata in mice, resulting in performance deficits in various behavioral tasks used to assess learning and memory. In addition to this selective effect, TMT also causes systemic toxicity which is manifested by malaise, cessation of eating and drinking, and death in severe cases. Four-month-old male mice from four inbred strains, AKR/J, BALB/cByJ, C57BL/6J and DBA/2J, were compared for their relative sensitivities to the toxic effects of TMT following a single i.p. injection of TMT chloride at 1.5, 1.8, 2.0, 2.3, 2.5, 2.7 and 3.0 mg/kg. The systemic toxicity of TMT was assessed using body weight changes, clinical signs and mortality. Behavioral effects were assessed through Morris water maze swim performance and passive avoidance test. Neurohistopathological effects were determined through silver staining for neurodegeneration. TMT produced a dose- and strain-dependent decrease in body weight, ranking in severity as follows: AKR/J > BALB/cByJ > (C57BL/6J = DBA/2J). Clinical signs were most severe in AKR/J mice and included hyperexcitability, tremors and tonic-clonic convulsions. The lethal dose of TMT was 3.0 mg/kg. The order of death was: C57BL/6J (< 24 h), AKR/J and DBA/2J (24–48 h) and BALB/cByJ (> 48 h). No behavioral or neurohistopathological changes due to TMT were seen in any strain at the dose levels tested, perhaps because the mice were too young to bring the neurotoxic range down into the sublethal systemic range. Toxicokinetic evaluation of TMT at 2.3 mg/kg revealed that blood levels of TMT were significantly lower ($p < 0.001$) and volume of distribution greater ($p < 0.0001$) in C57BL/6J mice than other strains.

1665 ERG CHANGES IN RATS EXPOSED TO TRIMETHYLTIN

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Trimethyltin (TMT) is an organotin known to be neurotoxic to many structures in the central nervous system. We measured electroretinograph (ERG) changes in male and female Sprague Dawley rats administered vehicle, 3, 6, or 9 mg/kg of TMT chloride as a single dose via oral gavage. ERG changes were assessed using the UTAS 2000 ERG measurement system. Needle electrodes were placed at the lateral canthus and in the sclera near the limbus of both eyes. A protocol was developed to measure a-wave, b-wave, c-wave, oscillatory and flicker potentials. Amplitudes were generally lower in the left eyes compared to the right eyes. Effects of treatment were more pronounced in the right eyes compared to the left. A-wave amplitudes were significantly lower than vehicle controls in rats exposed to 9 mg/kg TMT (−139.2 +/− 61.6 vs. −67.6 +/− 35.9 mV, control vs. treated, mean & sd). The amplitude of the second oscillatory potential was also lower in the high dose group (75.7 +/− 31.6 vs. 32.6 +/− 10.8 mV). The amplitudes of flicker potentials measured at 20, 25 and 30 Hz were all lower in the high dose group compared to vehicle controls. None of the measured endpoints in the 3 and 6 mg/kg dose groups were significantly different from vehicle controls. These data indicate that rats receiving a single dose of 9 mg/kg of TMT show measurable changes in ERG amplitudes 6 weeks after exposure. Changes in sensory systems should be considered when assessing the effects of TMT on cognitive or behavioral tests that rely on visual cues.

1666 NEUROTOXICITY OF INTRATHECAL MANGANESE^{2+} IN THE RAT

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We investigated the inhibition of motor activity in young male Sprague-Dawley rats (225 g) and the time course of Mn accumulation and dopamine depletion after the intrathecal administration of $MnCl_2$. One day after the intrathecal administration of 1 or 2 mg Mn^{2+}/kg, motor activity (n = 5 rats) was decreased to 10.6% or 8.9% of pre-manganese activity, respectively. Control animals (equimolar Cl-, n = 5 rats) were unaffected. Mn levels in the caudate-putamen, ventral masencephalon, occipital pole, frontal cortex and cerebellum were determined at 3, 6, 12, 24, 48, 72 and 120 hours after intrathecal Mn2+ (250 μg Mn/rat as $MnCl_2$, n = 4 rats) by graphite furnace atomic absorption. Six hours post-intrathecal Mn, the ventral mesencephalon had the largest mount of Mn (59.3 μg Mn/g); the next largest amount was in the occipital pole (12.1 μg Mn/g). The caudate-putamen gradually accumulated Mn with a maximum level at 120 hours post-injection (6.1 μg Mn/g). Dopamine, DOPAC, HVA and serotonin levels in the caudate-putamen and ventral mesencephalon were determined by HPLC-ECD at 3, 6, 12, 24 and 120 hours after intrathecal Mn^{2+} (n = 3 rats). Dopamine levels in the caudate-putamen at 6 and 120 hours were 52% and 41% of controls, respectively. Dopamine, DOPAC and HVA were not different from controls in the ventral mesencephalon. Serotonin levels in the caudate-putamen and the ventral mesencephalon were unaffected by intrathecal Mn^{2+}. (Supported in part by Superfund Basic Science Grant NIEHS #ES-04940.)

1667 CHROMIUM INDUCES A PERSISTENT ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) BY A PROTEIN KINASE C-INDEPENDENT MECHANISM

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Chromium is an important industrial metal and an environmental pollutant. Although the hexavalent form of chromium [Cr(VI)] is well established as a carcinogen, the paucity of epidemiological data involving human exposure has made it difficult to determine the manner by which this element acts to induce and/or promote the growth of tumors. The ability of this metal to cause various types of DNA lesions has supported the notion that chromium functions as an initiating agent in carcinogenic mechanisms. However, it is possible that some of the epigenetic effects of this element contribute to the induction of human neoplasms. We have found that Cr(VI) induces the tyrosine phosphorylation of several cellular proteins in rat hepatoma cells and alters the pattern of phosphoproteins produced in response to various growth factors. Furthermore, Cr(VI) induces the phosphorylation and (persistent) activation of MAPK, a key regulatory kinase in numerous growth factor signalling cascades. Many of the Cr(VI)-dependent effects were also observed in cells treated with the protein kinase C (PKC) agonist, phorbol 12-myristate 13-acetate (PMA). However, the bioeffects of Cr(VI) were determined to be independent of PKC activity since Cr(VI)-dependent phosphorylations/MAPK activation were observed in PKC-depleted cells. The ability of Cr(VI) to influence phosphoprotein production and MAPK activity in this manner suggests a mechanisms of

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