

Metallothionein and Cadmium Nephrotoxicity

Abstracts of Poster Session

Multiple Mechanisms are Involved in Cellular Response to Cadmium: Metallothionein Gene Organization and Regulation and Other Factors. C. E. HILDEBRAND, B.D. CRAWFORD, M. D. ENGER, B. B. GRIFFITH, J. K. GRIFFITH, J. C. SEAGRAVE, J. G. TESMER, R. A. TOBEY and R. A. WALTERS, *Genetics and Toxicology Groups, Los Alamos National Laboratory, Los Alamos, NM 87545.*

Combined mammalian somatic cell and molecular genetics approaches have been used to define mechanisms which modulate cellular sensitivity or resistance to cadmium (Cd). A series of cadmium-resistant (Cd^r) clonal cell lines has been derived from the Cd-sensitive (Cd^s) Chinese hamster (CHO) cell. These cell lines display threshold levels for CdCl₂ toxicity ranging from 0.2 μM (Cd^s CHO cell) to 200 μM (Cd^r200T1 cell) in monolayer culture.

A major factor in expression of the Cd^r phenotype is the ability of cells to induce synthesis of metallothionein (MT). The Cd^s CHO cell does not produce detectable MT in the absence or presence of Cd²⁺. Conversion of the CHO cell to Cd^r cell line correlates with a switch from noninducibility to inducibility of MT synthesis. Treatment of CHO cells with a DNA hypomethylating agent (5-azacytidine) increased the frequency of a phenotype switch from Cd sensitivity to Cd resistance. Molecular genetic analyses of genomic DNAs from Cd^s and Cd^r cells using molecularly cloned cDNA sequences encoding Chinese hamster MTI and MTII confirmed that the switch from MT noninducibility to MT inducibility involves changes in the pattern of DNA methylation in the region of the MT genes.

Increased levels of Cd-resistance are related to overproduction of MT. Molecular genetic analyses of genomic DNAs from both CHO and the sublines resistant to high Cd concentrations revealed coordinate amplification of both MTI and II genes up to 14-fold above the number of MT gene copies in the Cd^s CHO cell.

In addition to the involvement of the MT gene family in cellular responsiveness to heavy metals, other domains of cellular response have been identified. These domains include altered thiol metabolism following cellular Cd exposure as well as modulation of expression of multiple cytoplasmic non-MT proteins. Studies of the roles of these responses in Cd metabolism are in progress.

The Use of a Metallothionein I cDNA Probe for Quantitating Changes in MT-1 mRNA Levels in Maternal and Fetal Tissues of the Mouse. C. J. QUARFIE, and N. K. MOTTET, *Department of Pathology, University of Washington,* and D. M. DURHAM and R. D. PALMITER, *Department of Biochemistry, University of Washington, Seattle 98195.*

Metallothionein (MT) protein levels are elevated in the fetal liver. To study the mechanism of its induction, we prepared a cDNA probe to monitor changes in MT-1 levels. To prepare the probe, cloned sequences containing the coding region for MT-1 were nick-translated in the presence of ³²P-deoxynucleotides and the strands separated chromatographically to yield a single-stranded cDNA. MT-1 mRNA levels were measured in a solution hybridization assay in which the labeled cDNA anneals with MT-1 mRNA present in a total nucleic acid sample. As little as 0.5 pg mRNA can be detected within a day of total nucleic acid isolation.

We examined the temporal relationship between changes in the levels of plasma corticosterone, induction of fetal MT-1 mRNA and changes in zinc and copper concentrations. Our results show that fetal liver MT-1 mRNA levels become elevated during gestation (peaking on day 18) and that changes in concentration are predicted by changes in maternal and fetal plasma corticosterone levels. Placenta, maternal kidney and fetal kidneys (examined on day 18) did not respond similarly. Zinc was bound to fetal liver MT during the early induction phase, however, Cu became the predominant metal associated with MT at later times.

As a working hypothesis, we propose that corticosterone induces MT-1 mRNA in fetal liver and that resulting MT initially binds Zn which is subsequently displaced by copper.

Characterization of Bismuth- And Mercury-Induced Metallothioneins, J. A. SZYMANSKA*, M. J. STILLMAN, A. J. ZELAZOWSKI and J. K. PIOTROWSKI, *Department of Toxicological Chemistry Medical Academy, Lodz, Poland.*

We have shown previously that metallothioneinlike proteins can be isolated from rat livers and kidneys following injections of bismuth and mercury.

Optical absorption, circular dichroism, magnetic circular dichroism and emission spectra have been obtained for hepatic and renal proteins isolated after exposures of rats to BiCl_3 and HgCl_2 . Our results suggest that the liver proteins are zinc metallothioneins, whereas the renal MTs contain copper and metal-stimulator.

Spectroscopic Studies of the Metal Binding Sites in Metallothioneins. A. Y. C. LAW, J. A. SZYMANSKA and M. J. STILLMAN, *Department of Chemistry, The University of Western Ontario, London, Canada, N6A 5B7.*

Optical absorption, circular dichroism, magnetic circular dichroism and emission spectra have been obtained from a variety of Cd,Zn and Cd,Cu-metallothioneins. These data arise from chromophores at the metal ion binding sites in the MT and allow a view of chemical changes that take place at these binding sites.

Metal Substitution in Metallothioneins. A. Y. C. LAW, J. A. SZYMANSKA and M. J. STILLMAN, *Department of Chemistry, The University of Western Ontario, London, Canada, N6A 5B7.*

Substitution of the cadmium and zinc in Cd, Zn-MT with copper, mercury, cadmium and silver results in well-defined spectral changes. These can be associated with the initial direct replacement of the zinc, followed by replacement of the cadmium. Finally, at higher concentration, metal binding results in the loss of the stereochemical arrangement of sulfide-containing groups in the binding sites that is observed in the native protein.

Physicochemical and Metabolic Properties of Modified Metallothioneins. D. M. TEMPLETON and M. G. CHERIAN, *Department of Pathology, University of Western Ontario, London, Ontario, Canada N6A 5C1.*

The biocomplexes of cadmium may play an important role in the tissue deposition and toxicity of this metal. Injection of experimental animals with Cd^{2+} salts results in major deposition of Cd initially in the liver, whereas injected Cd-metallothionein (Cd-MT) is accumulated mainly in the renal tubules. Various organic chelates of Cd show an intermediate pattern of distribution. In order to better understand the factors controlling the binding of metals to MT, and Cd deposition and toxicity, we are investigating modified MTs as novel chelators of Cd.

Two distinct chemically crosslinked Cd-MT polymers have been prepared from rat liver Cd-MT-II. An octamer of MT (GA-MT) with a molecular weight of 53,000 was prepared by reaction with glutaraldehyde followed by NaBH_4 reduction. The polymerization resulted in modification of seven of nine lysine residues, but unaltered thiol content as shown by amino acid analysis and mercurial titrations. However, two of the tetracoordinate Cd binding sites (per monomer) were lost, indicating altered binding cluster geometry. The isoelectric point of GA-MT is increased to 5.2 from that of monomer (4.6). By reaction with dimethyl suberimidate, polymers (DMS-MT) have been prepared with molecular weights up to 100,000. DMS-MT has an unchanged isoelectric point (4.6) and similar

Cd binding sites to MT, with modifications of four lysines per monomer. Both types of polymer have lower frictional coefficients than the monomer. Phenyl mercuric derivatives of MT-II, GA-MT, and DMS-MT were prepared, which resulted in opening of the metal binding clusters. At phenyl mercuric substitution of ten or more thiols per monomer, all species are insoluble at their pI. Biphasic reactivity of the thiols with DTNB was observed for all species, which is consistent with proposed metal cluster structures.

When polymers labeled with ^{109}Cd are injected IV into rats, they stay in circulation longer than any other known soluble complex of Cd. A unique, uniform tissue distribution of Cd from the polymer was observed. The modifications of MT can affect both its metal binding properties and the metabolic fate of Cd.

Effects of *N,N*-Disubstituted Dithiocarbamates on Distribution and Excretion of Cadmium (Cd). G. R. GALE, *Veterans Administration Medical Center, and Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29403*, E. M. WALKER, JR., *Department of Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29403* and M. M. JONES, *Department of Chemistry and Center in Toxicology, Vanderbilt University, Nashville, TN 37235.*

N,N-Diethylthiocarbamate (DEDIC) is an antagonist of the acute toxicity of CdCl_2 in mice and is more effective than diethylenetriaminepentaacetate (DTPA) or dimercaptosuccinate (DMSA) in mobilizing Cd from its metallothionein-bound sites in liver, kidney and spleen. However, it promotes moderate elevations of Cd in lung, testes and heart, and over a 10-fold increase in brain Cd levels. The redistribution to brain has been attributed to a high octanol/water partition coefficient of the Cd complex with DEDIC, $\text{Cd}(\text{DEDIC})_2$, which has no polar group. Consequently, *N,N*-dihydroxyethylthiocarbamate (DHDC) and *N,N*-dicarboxymethylthiocarbamate (DCDC) were synthesized and compared with DEDIC for relative efficacies in mobilizing Cd from various tissues. DHDC effectively mobilized Cd from liver, kidney, and spleen, while not promoting its accumulation in lung, testes, heart, or brain. The DCDC was totally ineffective. DHDC also promoted Cd mobilization from bone. Unlike DEDIC, which promotes accumulation of Cd in skin and muscle, DHDC significantly reduced Cd levels in those tissues. The relative efficacies of the three analogs in reducing whole-body ^{109}Cd burden were $\text{DEDIC} > \text{DHDC} > \text{DCDC}$. Mobilization and excretion of Cd by these chelators were closely correlated with the octanol/aqueous partition coefficients of the Cd-chelator complexes.

Effects of NiCl_2 Treatment on Metallothionein Concentrations in Rat Liver and Kidney. F. W. SUNDERMAN, JR. and C. FRASER, *Departments of Laboratory Medicine and Pharmacology, University of Connecticut School of Medicine, Farmington, CT 06032.*

The effects of NiCl_2 treatment upon metallothionein (MT) concentrations were studied in livers and kidneys of groups of

five to eight male Fischer-344 rats, based upon MT-analysis by the Cd saturation-hemolysate procedure of Onosaka and Cherian. At 6.5 hr after an injection of NiCl_2 (0.75 $\mu\text{mole/kg}$, SC), MT concentrations averaged $125 \pm 49 \mu\text{g/g}$ in liver and $174 \pm 32 \mu\text{g/g}$ in kidney, ($p < 0.02$ vs. corresponding values of 49 ± 15 and 136 ± 6 in vehicle controls). At 17 hr after injection of NiCl_2 (0.75 $\mu\text{mole/kg}$, SC), MT concentrations averaged $318 \pm 58 \mu\text{g/g}$ in liver and $276 \pm 75 \mu\text{g/g}$ in kidney, ($p < 0.001$ vs. corresponding values of 39 ± 10 and 120 ± 8 in vehicle controls). Dose-effect relationships were observed for NiCl_2 -stimulation of MT concentrations in liver and kidney. For example, at 17 hr after intermediate NiCl_2 dosages (0.25 and 0.50 $\mu\text{mole/kg}$, SC) MT concentrations averaged 156 ± 38 and $226 \pm 23 \mu\text{g/g}$ in liver, and 239 ± 49 and $246 \pm 32 \mu\text{g/g}$ in kidney ($p < 0.001$ vs. vehicle controls). Induction of MT concentrations in liver and kidney at 17 h after NiCl_2 treatment (0.25 $\mu\text{mole/kg}$, SC) was not prevented by IP actinomycin D (0.5 mg/kg at 18 hr, 0.25 mg/kg at 16 hr, and 0.25 mg/kg at 14 hr before death). Repeated administration of NiCl_2 (0.10 $\mu\text{mole/kg}$, IP, on four successive days, with sacrifice 3 days after the last treatment) caused slightly increased MT concentrations (liver MT = $66 \pm 19 \mu\text{g/g}$ in liver and 175 ± 30 in kidney of NiCl_2 rats, ($p < 0.05$ vs. corresponding values of 46 ± 9 and 138 ± 23 in vehicle controls). At the same dosage and treatment schedule, CdCl_2 produced greatly increased MT concentrations (liver MT = $752 \pm 226 \mu\text{g/g}$; kidney MT = $428 \pm 169 \mu\text{g/g}$).

Is Cadmium Released from Metallothionein in Kidneys Preserved for Transplantation?

C. G. ELINDER, B. PALM and M. PISCATOR, *Department of Environmental Hygiene, Karolinska Institute, and The National Institute of Environmental Medicine, S-104 01 Stockholm, Sweden*, G. LUNDGREN, *Department of Transplantation Surgery, Huddinge Hospital, S-141 86 Huddinge, Sweden*, and M. NORDBERG, *Department of Environmental Medicine, Umeå University, S-901 87 Umeå, Sweden*.

Thirteen rabbits were given repeated cadmium injections to achieve cadmium concentrations in kidney cortex ranging from 0.05 to 1 $\mu\text{mole Cd/kg}$ wet weight. Another four animals served as controls. One kidney from each animal was frozen directly to -70°C , whereas the other kidney was kept for 24 hr at $\pm 0^\circ\text{C}$ in a preservative (Sachs' solution) to simulate conditions for preservation of human donor kidneys before transplantation. Protein binding of cadmium, zinc, and copper in kidney homogenates and the concentration of metallothionein (MT) was measured in the kidney that was frozen directly and in the kidney that had been preserved.

No gross differences in the protein binding of cadmium, zinc and copper or in the metallothionein content were seen between the directly frozen and preserved kidneys from the same animal. This indicates that MT is not rapidly broken down in rabbit kidneys which have been preserved similarly to human donor kidneys 24 hr prior to a transplantation in a proper standard preservation solution.

Uptake and Distribution of Cadmium in Rats Intubated with Rat and Crab Metallothioneins. M. A. WIEDOW and J. M. FRAZIER, *Division of Toxicology, School of Hygiene and Public*

Health, Johns Hopkins University, Baltimore, MD 21205.

The role of different animal metallothioneins (MT) and cadmium (Cd) concentrations on the uptake and distribution of Cd in rats was studied. Adult male (500–600 g) Wistar rats ($N = 24$) were divided into four exposure groups. Animals were maintained in metabolic cages and fed *ad libitum*. Each group was intubated with 3 mL of a 0.9% saline solution containing cadmium and ^{109}Cd tracer. The first three groups were given 60 $\mu\text{g Cd}$ either in the ionic salt form (0.5 μCi) or one of the Cd-bound proteins (crab MT, 0.5 μCi ; rat MT-II, 0.2 μCi). Animals were sacrificed by exsanguination 24 hr after exposure. Urine, feces, target (liver and kidney) and nontarget tissues (lung, heart, pancreas, spleen, bone, muscle, testis, blood and washed intestine and stomach) were monitored for ^{109}Cd activity. There were detectable levels of ^{109}Cd in target tissues, but $>98\%$ of the activity was confined to the intestine, digested material and feces. Cd was not detected in the urine or any of the nontarget tissues. The average uptake of Cd from the metal-bound crab and rat protein groups were 0.13 and 0.11%, respectively. However, the uptake in the ionic salt treated animals were 1.35% for the 60 $\mu\text{g Cd}$ exposure and 0.05% in the low Cd-dosed group. The kidneys from the MT-exposed animals contained 41.0 ± 4.6 (crab) and $46.3 \pm 4.5\%$ (rat) of the administered Cd dose, but only $9.0 \pm 1.3\%$ of the Cd distribution was located in this tissue for the high dosed Cd rats. The low dose Cd exposure group had $24.1 \pm 6.6\%$ of the absorbed activity in the kidney. These data indicate that a high dose of ionic Cd is more rapidly taken up by the gut in the rat, but Cd presented in a low dose or especially as MT may influence the distribution of the metal towards the kidneys. Further studies on intermediate doses of Cd are presently being conducted.

A Case-Control Study of Environmental Factors and Chronic Renal Failure. D. P. SANDLER, *National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

There are currently more than 50,000 individuals in the U.S. on maintenance dialysis for end-stage renal disease at a cost of more than \$1.5 billion per year. Individuals on dialysis represent only part of the population with chronic renal failure. Chronic renal failure (CRF) is a significant public health problem, but its etiology is poorly understood. Epidemiologic study of CRF has been made difficult by lack of uniform criteria for diagnosis or classification of patients. In addition, chronic renal failure often develops slowly over a number of years, making it difficult to recognize, in retrospect, an etiologic agent or agents.

A number of environmental agents have been implicated, nonetheless, in the etiology of CRF. These include lead, cadmium, analgesic drugs and solvents. For some of these factors, the evidence is fairly convincing that they play a role in the development of CRF. For others, the evidence is less well documented. Few controlled studies of the role of any potential risk factors in accounting for CRF incidence has yet to be determined.

Our study will evaluate the role of environmental factors in the etiology of chronic renal failure. Using a case-control interview approach, we will determine the frequency with which certain exposures occur prior to the development of CRF and compare the frequency of such exposures to that in individuals without renal disease. We intend to evaluate the

relative importance of suspected renal disease risk factors and to examine interactions between exposures. By including a wide range of CRF patients in the study, we will be able to examine whether risk patterns vary with disease severity or with histopathologic diagnosis.

Saturation Analysis and Copper Displacement Studies on the Binding Site of the Cadmium-binding Protein from the American Oyster (*Crassostrea virginica*). P. MISTRY, C. L. CZOP, D. P. ELLIOTT, C. F. CHIGNELL and B. A. FOWLER. *National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Previous studies have shown that the American oyster (*Crassostrea virginica*) produces a low molecular weight cadmium-binding protein (CdBP) similar in size to metallothionein (MT) but which contains less cysteine and binds only 1–2 g-atoms Cd/mole protein. Scatchard analysis of ^{109}Cd binding to purified CdBP showed a single class of site(s) with an apparent dissociation constant (K_d) of 10^{-7}M for Cd. Addition of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) to CdBP followed by separation of protein bound and free ionic Cd on small Sephadex G-25 columns demonstrated displacement of Cd from CdBP. A variable SH:Cd ratio of 4.5:1 to 2:1 for CdBP depending upon Cd saturation status instead of the 3:1 ratio reported for MT was determined by this method. Incubation of CdBP with EDTA (1.3 mM) showed little release of Cd from the protein except when DTNB was added. Circular dichroism studies of CdBP incubated *in vitro* with a 2-fold excess of Cd or Cu disclosed marked reduction in the positive 259 nm Cd-S bond peak but no changes in other portions of the spectrum. In addition, CdBP isolated from oysters collected in areas with greater human activity and possessing higher tissue burdens of Cu from *in vivo* exposure showed similar circular dichroic properties. These studies suggest that the lower Cd-binding affinity of CdBP relative to MT stems from the presence of only 2 SH groups per Cd at saturation, but that like MT these groups inhibit EDTA chelation of Cd from the protein. Addition of excess Cd or Cu resulted in the formation of an optically less active complex, at 259 nm.

Metal-binding Proteins in the American Oyster: Effects of Cadmium and Copper. D. W. ENGEL, *National Marine Fisheries Service, NOAA, Southeast Fisheries Center, Beaufort Laboratory, Beaufort, NC 28516.*

The American oyster, *Crassostrea virginica*, has a demonstrated ability to accumulate trace metals such as copper,

cadmium and zinc to very high concentrations without detectable physiological changes. This mollusc also produces copper and cadmium-binding proteins when exposed to these metals under laboratory conditions. In the current set of experiments oysters were: (Experiment 1) exposed to cadmium, 0.1 ppm, for 4 weeks and then allowed to depurate for 4 weeks in the absence of cadmium and (Experiment 2), exposed to cadmium and copper, 0.1 and 0.025 ppm for 4 weeks and then allowed to depurate for 4 weeks in the absence of cadmium but in the presence of elevated copper. Exposed oysters were sampled weekly for 8 weeks and analyzed for total copper, cadmium and zinc, and intracellular metal-binding proteins. Total metals were analyzed by atomic absorption spectrophotometry and the metal-binding proteins were separated on Sephadex G-75 gel-filtration media. In Experiment 1, cadmium displaces copper from the metal-binding proteins during the accumulation phase and in Experiment 2, copper displaced cadmium during the depuration phase. From mass balance calculations the percentage of the oyster cadmium on the metal-binding proteins ranged from 25 to 50% in Experiment 1 and from 12 to 60% in Experiment 2, but the forcing functions and time courses were different. The percentage of protein bound copper, however, in both experiments was about 30% throughout the experiments. Further, mass balance equations indicate that the metal-binding proteins in the oysters are primarily copper proteins that can be activated by elevated levels of cadmium.

Monoclonal Antibodies to Metallothionein from Cd²⁺-Resistant Chinese Hamster Lung Fibroblasts. T. MASUI and T. UTAKOJI, *Department of Cell Biology, Cancer Institute, Toshima-ku, Tokyo 170, Japan,* and M. KIMURA, *Department of Experimental Toxicology, National Institute of Industrial Health, Tama-ku, Kawasaki 213, Japan.*

Four monoclonal antibodies of the mouse against metallothionein-2 (MT-2) from Cd-resistant fibroblasts of the Chinese hamster lung (Cd^r-CHL) have been prepared. Each one of the antibodies showed a unique reactivity pattern against MTs of several mammals. On the contrary, polyclonal antisera of rabbit against native or polymerized mouse liver MT-2 showed general cross-reactivity.

Reactivities of MTs with these monoclonal antibodies were affected by amino acid sequence, metal composition and other factors, such as the presence of β -mercaptoethanol.

Monoclonal antibodies will give us detailed information on the three-dimensional molecular configuration of the metallothioneins in various conditions.

Table 1.

Metallothionein	ACM-1a	ACM-2a	ACM-3a	ACM-4a	C-1a	C-2a	C-3a
Cdr-CHL MT-1	+	–	+	–	+	+	+
MT-2	+	+	+	+	+	+	+
Mouse-liver MT-1	+	–	±	–	+	+	+
(Cd-induced) MT-2	+	+	+	–	+	+	+
Rat-liver MT-1	+	–	–	±	+	+	+
(Cd-induced) MT-2	+	+	+	+	+	+	+
Rat-liver MT-1	+	–	–	–	+	+	+
(Zn-induced) MT-2	+	–	–	±	+	+	+

^aACM = ascitic fluids of mouse hybridomas; C = conventional antiserum of rabbit against mouse MT-2.

Monoclonal Antibodies to Metallothionein from Cd²⁺-Resistant Chinese Hamster Lung Fibroblasts. M. KIMURA, T. MASUI and T. UTAKOJI, *National Institute of Industrial Health, Ministry of Labour, Tama-ku, Kawasaki 123, Japan.*

Four monoclonal antibodies of the mouse against metallothioneins (Mts) from CD²⁺-resistant fibroblasts of the Chinese hamster lung (Cd-CHL) have been prepared. Each one of the antibodies showed a unique cross-reactivity pattern when tested against MT from the livers of several mammals and from yeast.

Effect of Chelating Agents on Cadmium Retention in Lung, Liver and Kidney of Rats After Inhalation Exposure. Y. H. LEE and G. OBERDOERSTER, *Division of Toxicology, University of Rochester, Rochester, NY 14642.*

Some chelating agents such as BAL and EDTA have been shown to reduce the mortality in animals with increased nephrotoxicity of Cd. Therefore, an increase in kidney Cd levels after chelating agent treatment should be avoided. Thus, we studied the effectiveness of BAL and DMPS given by different routes on mobilizing Cd in body organs after inhalation exposure of Cd. In all studies, Long-Evans male rats weighing 185–225 g (10 rats per group) were used and they were exposed to ¹⁰⁹CdCl₂ in a nose-only system. In the first experiment, BAL was given IP, 50 mg/kg/day in 1 mL propylene glycol, 5 days per week for 2 weeks, starting immediately after exposure to 38 µg/m³ ¹⁰⁹CdCl₂ for 1 hr. The controls were injected IP with 1 mL/kg propylene glycol. In the second experiment, rats were treated with 500 µmole/kg/day DMPS, IP in 2 mL saline, 5 days per week for 2 weeks after exposure to 128 µg/m³ ¹⁰⁹CdCl₂ for 90 min. The controls were given 2 mL/kg saline only. Neither of the treatments decreased the body burden of Cd on the basis of in vivo thoracic counts. Also, Cd distribution in lung, liver and kidney was not altered by these treatments. In the third experiment, rats were treated by nose only exposure to 250 mg/m³ DMPS for 30 min per day for 14 days after ¹⁰⁹CdCl₂ exposure to 90 µg/m³ for 90 min. The controls were sham-exposed in nose-only tubes for 30 min daily. Liver Cd increased significantly, twice as much as that of the controls. Cd content in the lung also decreased. The amount of Cd decrease in the lung is almost equal to the Cd

increase in the liver of the treatment group. Cd levels in kidneys of the treated rats did not differ from that of controls. In order to find out whether this was an early or late effect of Cd mobilization from the lung, DMPS via inhalation was given only once immediately after ¹⁰⁹CdCl₂ inhalation in rats. It was found that lung and liver Cd was not altered; however, kidney Cd was significantly increased in the treated group. These data seem to suggest that Cd can be mobilized from the lung with DMPS treatment via inhalation, but further studies are needed to clarify the influence of the treatment on Cd burden of the kidneys.

Role of Metallothionein in Ehrlich Cells: Cellular and Chemical Studies. A. KRAKER, S. KREZOSKI, G. BACHOWSKI, J. SCHNEIDER, C. F. SHAW III, J. D. OTVOS, and D. H. PETERING, *Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201.*

Ehrlich ascites tumor cells make constitutively Zn, Cu, Cd binding protein with the properties of metallothionein (MT). During host zinc deficiency, which inhibits Ehrlich cell proliferation, zinc is specifically lost from MT. Readdition of limited amounts of Zn to the diet stimulates cell growth and division without the return of Zn to MT. Results suggest that MT is a metabolically active form of zinc in these cells which may serve as an intermediate in the synthesis of holo-Zn proteins. In support of this view, MT is shown to be the predominant, perhaps, exclusive source of cytosolic Zn for the reconstitution of added apocarbonic anhydrase. Besides the kinetic reactivity of Zn in MT, the stability constants of zinc in its two-metal clusters are in the range of 10⁹–10¹⁰ mole. Both features support the dynamic nature of the Zn in MT. To investigate further the role of MT in Ehrlich cell proliferation, a cytotoxic copper complex, 3-ethoxy-2-oxobutylaldehyde bis(thiosemicarbazonato)Cu(II), was reacted with these cells. The complex is reduced and dissociated. In the range of growth inhibition, Cu(I) specifically displaces Zn from MT. Some copper binds to high molecular weight protein. The reactivation of DNA synthesis and cell division follows the rapid loss of Cu from the high molecular weight band and the subsequent return of Zn to MT. These results and similar ones using Cd²⁺ in place of copper are consistent with a key role of MT in cell proliferation, but do not exclude the possibility that other sites are involved.