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. Seag rave, 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 (CdR) clonal cell lines has been derived from the Cd-sensitive (CdS) Chinese hamster (CHO) cell. These cell lines display threshold levels for CdCl₂ toxicity ranging from 0.2 μM (CdS CHO cell) to 200 μM (CdR 200T1 cell) in monolayer culture.

A major factor in expression of the CdR phenotype is the ability of cells to induce synthesis of metallothionein (MT). The CdS CHO cell does not produce detectable MT in the absence or presence of Cd²⁺. Conversion of the CHO cell to CdR 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 CdS and CdR cells using molecularly cloned cDNA sequences encoding Chinese hamster MT-I and MT-II 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 MT-I and II genes up to 14-fold above the number of MT gene copies in the CdS 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. Quaife, and N. K. Mottet, Department of Pathology, University of Washington, and D. M. Durnam and R. D. Palmier, 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-translation 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 metallothionein-like 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 BiCl₃ and HgCl₂. 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 Cd²⁺ 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 NaBH₄ 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 tetracordinate 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 ¹⁰⁹Cd 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 37233.

N,N-Diethylidithiocarbamate (DED) is an antagonist of the acute toxicity of CdCl₂ in mice and is more effective than diethylentriaminepentaacetic (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 DEDC, Cd(DED)₂, which has no polar group. Consequently, N,N-dihydroxyethylidithiocarbamate (DHDC) and N,N-dicarboxymethylidithiocarbamate (DCDC) were synthesized and compared with DEDC 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 DEDC, which promotes accumulation of Cd in skin and muscle, DHDC significantly reduced Cd levels in these tissues. The relative efficacies of the three analogs in reducing whole-body ¹⁰⁹Cd burden were DEDC > DHDC > 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 NiCl₂ 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 NiCl₂ 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 NiCl₂ (0.75 µmol/kg, SC), MT concentrations averaged 125 ± 49 µg/g in liver and 174 ± 32 µg/g in kidney, (p < 0.02 vs. corresponding values of 49 ± 15 and 136 ± 6 in vehicle controls). At 17 hr after injection of NiCl₂ (0.75 µmol/kg, SC), MT concentrations averaged 318 ± 58 µg/g in liver and 276 ± 75 µg/g in kidney, (p < 0.001 vs. corresponding values of 39 ± 10 and 120 ± 8 in vehicle controls). Dose–effect relationships were observed for NiCl₂ stimulation of MT concentrations in liver and kidney. For example, at 17 hr after intermediate NiCl₂ dosages (0.25 and 0.50 µmol/kg, SC) MT concentrations averaged 156 ± 38 and 226 ± 23 µg/g in liver, and 239 ± 49 and 246 ± 32 µg/g in kidney (p < 0.001 vs. vehicle controls). Induction of MT concentrations in liver and kidney at 17 hr after NiCl₂ treatment (0.25 µmol/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 NiCl₂ (0.10 µmol/kg, IP, on four successive days, with sacrifice 3 days after the last treatment) caused slightly increased MT concentrations (MT = 66 ± 19 µg/g in liver and 175 ± 30 in kidney of NiCl₂ rats, (p < 0.05 vs. corresponding values of 46 ± 9 and 138 ± 23 in vehicle controls). At the same dosage and treatment schedule, CdCl₂ produced greatly increased MT concentrations (liver MT = 752 ± 226 µg/g; kidney MT = 428 ± 169 µg/g).

Is Cadmium Released from Metallothionein in Kidneys Preserved for Transplantation? C. G. Elinen, 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 µmol 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 ±0°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 109Cd tracer. The first three groups were given 60 µg 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 109Cd activity. There were detectable levels of 109Cd 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 liver 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 was 1.35% for the 60 µg 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 ± 4.5% (rat) of the administered Cd dose, but only 9.0 ± 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 ± 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

Previous studies have shown that the American oyster
(Crassostrea virginica) produces a low molecular weight
cadmium-binding protein (CdBP) similar in size to metallothio-
ein (MT) but which contains less cysteine and binds only 1–2
atoms Cd/mole protein. Scatchard analysis of 109Cd binding
to purified CdBP showed a single class of site(s) with an
apparent dissociation constant (Kd) 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. Addi-
tion 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.
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The American oyster, Crassostrea virginica, has a dem-
strated ability to accumulate trace metals such as copper,
cadmium and zinc to very high concentrations without detect-
able physiological changes. This mollusc also produces copper
and cadmium-binding proteins when exposed to these metals
under laboratory conditions. In the current set of experi-
ments 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 cad-
mium 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 spectropho-
tometry and the metal-binding proteins were separated on
Sephadex G–75 gel-filtration media. In Experiment 1, cad-
mium displaces copper from the metal-binding proteins dur-
ing 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 Experi-
ment 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, Depart-
ment of Cell Biology, Cancer Institute, Toshima-
ku, Tokyo 170, Japan, and M. Kimura, Depart-
ment of Experimental Toxicology, National
Institute of Industrial Health, Tama-ku, Kawa-
saki 213, Japan.

Four monoclonal antibodies of the mouse against metallo-
thein-2 (MT-2) from Cd-resistant fibroblasts of the Chine-
sese hamster lung (Cd-CHL) have been prepared. Each one of
the antibodies showed a unique reaction 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 metal-
thioneins in various conditions.

Table 1.

| Metallothionein   | ACM-1a | ACM-2a | ACM-3a | ACM-4a | C-1a | C-2a | C-3a |
|-------------------|--------|--------|--------|--------|------|------|------|
| Cd-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 | +      | −      | −      | +      | +    | +    | +    |

*ACM = ascitic fluids of mouse hybridomas; C = conventional antiserum of rabbit against mouse MT-2.
Monoclonal Antibodies to Metallothionein from Cd\(^{2+}\)-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 Cd\(^{2+}\times\)-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 14624.

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 \(^{109}\)CdCl\(_2\) 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 \(\mu\)g/m\(^3\) \(^{109}\)CdCl\(_2\) for 1 hr. The controls were injected IP with 1 mL/kg propylene glycol. In the second experiment, rats were treated with 500 \(\mu\)mole/kg/day DMPS, IP in 2 mL saline, 5 days per week for 2 weeks after exposure to 128 \(\mu\)g/m\(^3\) \(^{109}\)CdCl\(_2\) 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/m\(^3\) DMPS for 30 min per day for 14 days after \(^{109}\)CdCl\(_2\) exposure to 90 \(\mu\)g/m\(^3\) 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 \(^{109}\)CdCl\(_2\) 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. Reduction 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 \(10^{9}–10^{10}\) 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-oxobutyraldehyde bis(thiosemicarbazonato)Cu(II), was reacted with these cells. The complex is reduced and dissociated. In the range of growth inhibition, Cu(I) specifically displaced 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 Cd\(^{2+}\) 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.