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Discussion Summary. Roles of Metallothionein and Related Proteins in Metal Metabolism and Toxicity: Problems and Perspectives

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This summary examines some of the known and hypothesized roles of metallothionein and related proteins in mediating the metal metabolism and toxicity from a chemical perspective. It attempts to examine in kinetic terms how such molecules may exert homeostatic control over the intracellular bioavailability of metal ions to essential enzymatic or other molecular systems. The concept of ongoing competition between metallothionein and related proteins with other intracellular metal-binding sites for various metals is also examined in relation to the thermodynamic stability of these proteins. Comparisons between mammalian metallothionein and analogous nonmammalian proteins demonstrate both similarities and great differences in types of metal-binding sites, metal-binding constants, amino acid composition, and secondary structures such that apparent diversity of these low molecular weight metal-binding molecules in nature appears to be growing ever wider. The potential value of these data rests both in delineating new hypotheses for metallothionein evolution and in suggesting new model systems for discovering the normal function of metallothionein and related proteins in cells.

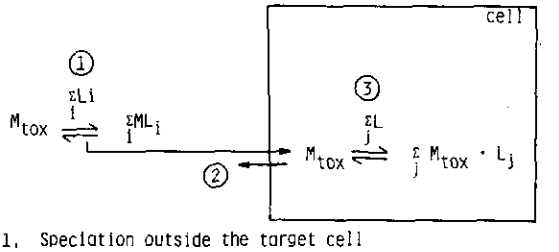
Introduction

It has been 27 years since Margoshes and Vallee isolated a cadmium-binding protein which they called metallothionein (*1*). In the intervening period this protein has become a major subject for study by metalloprotein chemists, toxicologists, molecular biologists, and others; yet the functions of metallothionein in the metabolism of normal and toxic metals are still formidable problems. The preceding papers of this symposium suggest that once one moves beyond mammals to other classes of living systems, there is a large diversity of cadmium-binding proteins. Thus, although the perspective developed in this paper on the function of nonmammalian metal-binding proteins in essential metal metabolism and cadmium toxicity draws its roots from studies of metallothionein, it now appears that each of these nonmammalian systems must be examined individually without assuming that the metal-binding proteins closely resemble metallothionein in structure or chemistry.

The approach of this paper will be to provide a conceptual picture of possible mechanisms of Cd toxicity based on studies of mammalian metallothionein and to weave in aspects of the previous papers of this symposium which relate to function and toxicity of the nonmammalian metal-binding protein. This information was taken directly from oral presentations and is accurate in overall character and hopefully in detail. However, the reader is directed to the specific papers for firsthand information.

Metal Toxicity as a Conceptual Problem

The chemistry and biochemistry of metal toxicity may conveniently be divided into three subjects according to Figure 1: extracellular speciation, uptake, and intracellular reactions. Outside the organism or cell, toxic metals (M_{tox}) interact with a large number of metal chelating



1. Speciation outside the target cell
2. Uptake
3. Reactions in cells -- biochemical basis of toxicity

FIGURE 1. The problem of metal toxicity: general sites for study.

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structures to form an array of metal–ligand complexes, ML_i, the concentration of each depending on kinetic and thermodynamic features of the reactions involved. In order to have its effect, at least one species of the metal must be able to diffuse or be transported into the target cell. Once in the cell, M_{tox} again distributes itself among metal binding sites within the cell to form $M_{tox}{\cdot}L_j$. A thorough description of the reactions which M_{tox} and $M_{tox}{\cdot}L_j$ may undergo is provided elsewhere (*2*). However, for the present discussion of Cd^{2+}, the reactions in the cell will be analyzed [Eq. (1)].

$$\underset{\text{Species entering cell}}{M_{tox} \cdot L_i} + \underset{\text{Cellular metal-ligand sites}}{\sum_j (M)L_j} \underset{k_j}{\overset{K_{eq,j}}{\rightleftharpoons}} \sum_j M_{tox} \cdot L_j + (M) + L_i \quad (1)$$

In this reaction M_{tox} reacts with cellular constituents having metal binding affinity, which may or may not initially bind a biologically essential metal ion (M). The rate and extent of these reactions depends on k_j and $K_{eq,j}$, the rate and equilibrium constants for the reactions. The experimental questions to be addressed are (1) which reactions and sites are preferred and (2) how do such reactions participate in metal toxicity? Or, what is the nature of $(M)L_j$ which reacts with M_{tox} and how does the binding of M_{tox} to L_j affect its normal function.

As a foundation for the examination of reaction (1), there are several principles or operating assumptions to keep in mind. (1) Metals are not metabolizable to other products as are organic xenobiotics. (2) Nonenzymatic coordination chemistry plays major role in intracellular chemistry of M_{tox}. (3) As metals titrate cellular sites, assume first-binding sites may be normal binding sites for metals. (4) Metalloenzymes have strong kinetic preference for their natural metal and do not readily exchange metals; metal-activated enzymes are more labile. (5) Once metals bind to sites, biological response such as induction of L_j may occur. (6) We know relatively little about biochemistry of essential and toxic metal metabolism! Of these, (1) and (2) stress the difference between toxic organic chemicals and metals. While organic xenobiotics are likely to be modified in specific ways by enzymes such as the cytochrome P-450 systems, metals generally undergo reactions analogous to their inorganic coordination chemistry. A reasonable third presumption is that some of the cellular sites which most effectively bind M_{tox} are specific sites designed to complex essential metal ions. If this is correct, one may find clear interactions of essential and toxic metal metabolism. Still, from all previous study of mammalian systems, there is no evidence that metalloenzymes exchange their native metals for M_{tox} *in vivo*. However, under zinc-deficient conditions bacterial alkaline phosphatase will incorporate Cd (*3*). Once M_{tox} binds to some site L_j, perturbations of its normal function in cells are likely, considering that heavy metals in general alter the properties of proteins and polynucleic acids (*4,5*). Such effects may also lead to cellular adaptations such as the induction of more L_j to compensate for its functional loss when $M_{tox}{\cdot}L_j$ forms. Finally, the last point emphasizes that the biochemistry of essential metals is not well understood, even in the case of iron metabolism, which has been intensively studied for years. In this context it is not surprising that the mechanisms by which nonessential toxic metals interact with cells are also poorly comprehended.

Role of Metallothionein in Cadmium Toxicity: Acute and Chronic Exposure

A summary of how metallothionein participates in the metabolism of Cd during acute exposure of mammalian organisms to the metal is shown schematically in Figure 2. This model is based on numerous studies and refers to events primarily in liver and secondarily in other tissues in which metallothionein may be induced, such as kidney. Cadmium enters the cell and is initially distributed among a number of sites, CdL_j (*6*). By an unknown mechanism the metal induces the *de novo* synthesis of mRNA for Mt–protein, which then leads to the synthesis of apometallothionein (*7*). Cadmium binds to metallothionein with an average log apparent stability constant of 15 at pH 7.4 and 25°C (*8*). Because of this large binding affinity for Cd, apoMt competes successfully with CdL_j for the metal and cadmium is gathered into metallothionein. As the protein biodegrades, cadmium, which is released, reinduces the synthesis of apoMt (*9*). A steady state is established between the rate of biosynthesis (k_1) and biodegradation (k_2 [CdMt]), such that most if not all of the Cd is bound to metallothionein. In this form it is apparently not toxic to cells (*10*). According to this model there are only two conditions under which cadmium may be toxic: before the initial induction of Mt when it binds

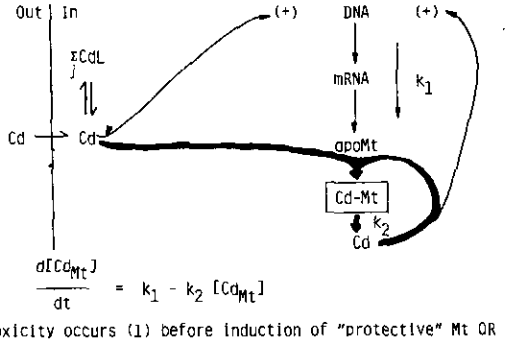

FIGURE 2. *The metabolism of cadmium in cells: relationship to toxicity.*

Table 1. Levels of cadmium and other metals used to elicit the formation of Cd-binding proteins in nonmammalian species.

Species	Metal-binding protein	Metal concentration
Pseudomonas putada	CdBP	3 mM Cd
Yeast	Cd-peptide	1 mM
Neurospora (natural role in asexual cycle)	Cu–Mt	Cu supplementation
Mushroom	Cd-mycophosphatin	10 μM
Alga	CdBP	50 μM
Rice	CdBP	30 μM
Mussel (*Mytilis edulis*)	CdBP	1 μM
	HgBP	5 μM
Whelk	CdBP	Natural exposure
Oysters	CdBP	Natural exposure
Cyanobacteria	CdBP	22 μM
Lobster	CdBP	89 ppm (food)
Crab	CdBP	Natural exposure
Drosophila	CdBP	15 μM
Carp	CdBP	1–15 ppm or 10–150 μM
Bluegill	CuBP	2 μM Cu (chronic)
Rainbow	Cd-Mt or CdBP	10–50 nM (nature of BP depends on route of administration)

to CdL_j and may perturb a variety of biochemical processes (*4*) and when so much cadmium has entered the cells and bound to Mt that k_2 [Mt] > k_1. In this situation the rate of biodegradation and release of Cd from the protein exceeds the rate of synthesis of apoMt and nonmetallothionein Cd complexes, CdL_j, form.

There are two shortcomings of this model. First, the mechanism by which non-Mt Cd causes toxicity is not known. Second, the model does not offer a mechanism by which chronic, low level toxicity may occur, for during such exposure neither condition occurs. There are documented effects of low concentrations of cadmium on liver, pancreas, fetus, neonate, and lung (*11–14*). In addition, it is noted that Figure 2 applies only to tissues such as liver and kidney which accumulate Cd. How Cd affects other tissues which are exposed to relatively minute levels, such as heart and lung, is not considered.

Two hypotheses to explain chronic toxicity of cadmium are variants of the mechanism in Figure 2. In one model, significant non-Mt-bound cadmium can exist in tissues even when relatively little of the metal enters the tissue. This could be because the tissue has a threshold to the induction of Mt–protein which is not reached and below which cadmium distributes itself among other sites after binding to basal metallothionein. Alternately, non-Mt Cd might coexist with induced levels of Cd, Zn-Mt, even though these levels are less than concentrations of protein needed to meet the condition in Figure 2 of k_2 [Mt] > k_1, invoked to explain the toxicity of large concentrations of Cd in tissues which readily synthesize metallothionein in response to exposure to the metal. Some evidence for each hypothesis exists in a rat kidney model for chronic

Table 2. Distribution of cadmium among cell components.

Species	Mt	Site HMW[a]	Other
Mammalian liver	95[b]	5	
Pseudomonas p	20	20	60, membrane
Neurospora C.	10 of extra copper		
Rice	70–80 in 7000 dalton band	20–30	
Mussel			
Cd	70–80 in 10,000 and 20,000 dalton bands	20–30	
Hg	70–80 in 10,000 and 20,000 dalton bands	20–30	
Whelk		Small percentage in cytosol	
Oyster	40	60	
Oyster	35 in 10,000 and 24,000 dalton bands		
Alga	80–90	10–20	
Cyanobacteria	>90		
Lobster	30	20	50, low molecular weight
Crab	>90		
Drosophila	>90		
Carp (2 mg/kg)	50	50	4-day exposure
	80	20	31-day exposure
Bluegill - Cu		Variable	
Rainbow	>90 (Mt)		Injection
	>90 (BP)		Cd in water

[a] HMW is high molecular weight fraction of cytosol isolated by Sephadex chromatography.
[b] Percentages of total.

cadmium distribution in zinc-normal and zinc-deficient animals (*15*).

Models for Cadmium Exposure in Nonmammalian Systems

Acute and chronic exposures involving large and small concentrations of cadmium represent different, important and real toxicological situations in both mammalian and nonmammalian systems. The reports of this meeting focused on the observation and characterization of metal-binding proteins (BP) which bind cadmium and other toxic metals (e.g., Hg, Cu). With some exceptions these papers did not indicate the toxic responses of the organisms to the exposure. Table 1 lists some of the approximate levels of metals to which organisms were exposed. Concentrations of metals in the exposure media ranged from 1 to 3000 μM Cd. Copper and mercury were also used as toxic metals.

In general, experimental conditions were chosen that elicited the formation of CdBPs. Large concentrations of metal were used relative to environmental levels (except in highly contaminated areas). Future studies will need to determine whether such proteins are made after lower levels of treatment with these metals. In each organism, discrete, low molecular weight metal-binding proteins were found after metal exposure. There was a general tacit assumption that these metal-binding proteins behave like mammalian metallothionein and probably protect the organism from metal toxicity. However, as discussed below, such an identification may not be warranted; nor is it clear that the binding of metal to BPs is a protective not a deleterious event.

The exposure regimens led not only to the binding of Cd to BP but in many cases to the distribution of Cd among other sites as well. One has the impression from a summary of distribution data (Table 2) that there is much more non-Mt bound Cd in a number of the nonmammalian organisms than found in rat liver or kidney (*15,16*). If so, one needs to reconsider the applicability of Figure 2 and its analysis as a basis for understanding cadmium toxicity.

Role of Zn(Cu)-Mt in Cells

Beyond the two hypotheses described above to account for the chronic, low level toxicity of cadmium, a third possibility will be explored in this paper, namely, that the binding of cadmium to basal metallothionein normally present in cells may produce toxicity (*2*). This suggestion arises out of a recognition that at least low levels of Mt exist in most mammalian tissues apart from any exposure of the organism to cadmium (*17*). Thus, one may suppose that the protein has normal functions to play in cells which might be disrupted when cadmium binds to it. From this perspective, therefore, one must know what such metabolic activities of Mt may be.

A diagram is shown in Figure 3 which summarizes the

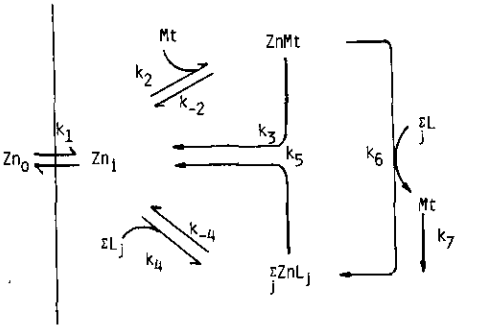

FIGURE 3. Metabolism of zinc in cells: role of metallothionein (1979).

view of the place of liver Zn-Mt in cellular zinc metabolism as it was understood in 1979. Besides Cd^{2+}, glucocorticoids and perhaps other mediators of the generalized stress response of mammals induce the synthesis of Mt (*18,19*). Excess zinc, itself, does this (*20*). Unlike Cd, however, it does not reinduce the synthesis of metallothionein as the metal is liberated during biodegradation (*20*). In this model, Zn-Mt is a chemically inert storage form of zinc, which becomes available only as the protein degrades. Underlying this view is the understanding that zinc bound to Mt is thermodynamically or kinetically stable to dissociation or ligand substitution reactions involving other zinc-binding sites in the cell (L), reactions (2) and (3), respectively:

$$\text{Zn-Mt} \underset{K_{eq}}{\overset{k}{\rightleftharpoons}} \text{Zn} + \text{Mt} \qquad (2)$$

$$\text{Zn-Mt} + \text{L} \xrightarrow{k} \text{Zn-L} + \text{Mt} \qquad (3)$$

Recent studies of the chemical properties of Zn-Mt have shown that the protein is much more reactive in ligand-exchange chemistry than previously thought. Some of the findings which support this conclusion are the following. Zinc-metallothionein is reactive with small ligands such as EDTA and NTA (nitrilotriacetate) in ligand substitution reactions (*21,22*). Similarly, Zn_7-Mt undergoes a facile, bimolecular ligand exchange with apocarbonic anhydrase ($k = 2 \times 10^3$/sec-M at 25°C) (*21*). Recent measurements of the apparent stability constant of Zn_7-Mt indicates that in both clusters each metal ion binds with an average apparent stability constant of about 10^{11} at 25°C and pH 7.4 (*8*). If one assumes that zinc metalloproteins have stability constants of 10^{10} or larger, these results suggest that Zn_7-Mt is kinetically and thermodynamically competent to carry out ligand substitution chemistry in cells and to donate zinc to apozinc proteins in cells.

Studies suggest that under metabolic demand situations, Mt-bound zinc is a labile pool of the metal in cells. For example, over a 2-week period under zinc-deficient conditions Zn is lost only from basal Zn,Cu-Mt in rat kidney as judged by Sephadex G-75 chromatographic analysis of organ supernatant (*15*). Other protein-bound Zn is unaffected. Similarly, zinc is lost only from Mt in

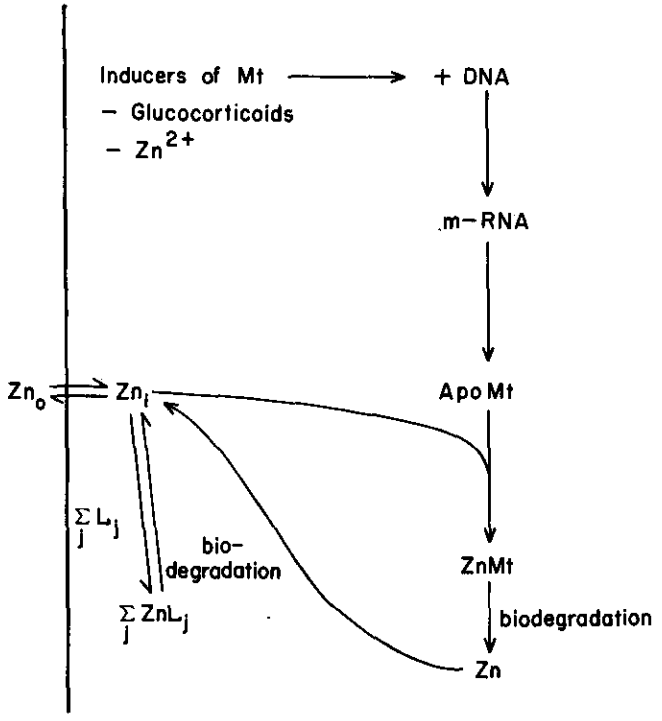

FIGURE 4. Hypothetical role of metallothionein in ligand substitution chemistry in cells.

Ehrlich cells injected into zinc-depleted mice (*23,24*). A related experiment with Ehrlich cell supernatant shows that when apocarbonic anhydrase is added to it, the only source of zinc to reconstitute active zinc carbonic anhydrase is metallothionein, again pointing to the unique metabolic reactivity of Zn in metallothionein (*24*).

Figure 4 diagrams a model of the role for Zn-Mt in cells in which the protein acts as an active donor of Zn to other sites in cells. If this is correct and the availability of Mt-Zn is not limited by the biodegradation rate of Mt (step k_3), then one will see metallothionein-zinc turn over faster than the protein in metabolic demand situations. This hypothesis was tested in Ehrlich cells labeled with ^{35}S-cysteine, which were placed in a zinc-deficient culture medium (*25*). Zinc is lost from Zn-Mt to other parts of the cell with a half time of 2 hrs. The protein turns over with a $t_{1/2}$ = 6–9 hr. Thus, in Ehrlich cells these results suggest that reaction (3) is occurring and that metallothionein is a chemically reactive donor of zinc to other cellular sites. Alternatively, the dissociation of zinc from Zn–Mt (step k_2) may be rate-limiting. However, this reaction is thought to be too slow to account for the observed kinetics of loss of Zn from Zn–Mt. If sustained by further studies, these experiments will link Zn–Mt for the first time directly to other cellular constituents and argue for a central role of Mt in basic cellular zinc metabolism, namely, in the constitution of holo-Zn-metalloproteins. A direct extension of this idea is that the binding of Cd to basal-Mt, below levels of exposure which induce extra Mt, may hinder the normal activity of this protein in ligand exchange chemistry [reaction (3)]. This possibility will be explored below.

The finding that metallothionein exists in a variety of mammalian tissues in the absence of heavy metal exposure or other obvious stimuli can be extended to nonmammalian species as well. Table 3 lists organisms which appear to have metallothionein or metal-binding protein prior to cadmium exposure. Some authors did not examine the unexposed organism for binding protein, so it is likely that this list may grow. Nevertheless, the presence of BP in a number of systems again raises the question of its normal function in cells. In the crab, for example, a CuBP has been characterized, which can serve as a donor of Cu to apohemocyanin. One may also ask, therefore, whether the binding of toxic metals such as Cd affects its normal activities.

Chemical Properties of Cd_7-Mt

The remarkable cellular property of Cd-Mt is that in mammalian tissues Mt so efficiently competes with other sites to bind a great majority of the Cd. To what features of its chemistry can this be attributed? Recent studies of its thermodynamic and kinetic properties have shown that there are two kinetic classes of metals in ligand substitution reactions of Cd_7-Mt with some small ligands such as nitrilotriacetate and bis(thiosemicarbazones):

$$\text{Cd-Mt} + \text{L} \rightleftharpoons \text{Zn} \cdot \text{L} + \text{Mt} \qquad (4)$$

The classes may be the structural clusters, which behave as cooperative units. The average apparent stability constant for each of the seven Cd ions distributed among two metal clusters is about 10^{15} at 25°C and pH 7.4, some three orders of magnitude larger than that for Zn binding to Mt (*8*). In an earlier study Cd_7–Mt was shown to be inert in reaction with EDTA (*21*), but a current investigation showed that both nitrilotriacetate (NTA) and 3-ethoxy-2-oxobutyraldehyde bis(N^4-dimethylthiosemicarbazone) were much more reactive (*22*). Hence, it is probably the thermodynamic stability of Cd-Mt not its kinetic unreactivity which prevents a wider distribution of Cd within cells.

When the properties of mammalian Cd-Mt and nonmammalian Cd-binding proteins are compared using data presented at the conference, one appreciates immediately that the relative uniformity of mammalian metallothionein gives way to a remarkable diversity of proteins (Table 4). The structural relationship of such proteins to

Table 3. Presence of easily measurable BP in untreated cells.

Organism	Protein
Neurospora	CuBP
Rice	?
Whelk	apoprotein
Oyster	CuBP
Bacteria	BP
Blue crab	Zn,CuBP
Drosophilia	BP
Carp	Mt
Bluegill	CuBP
Rainbow trout	Mt or BP

Table 4. Structural properties of CdBP.[a]

	Molecular weight	Metal and sulfhydryl information	Other
Mammalian Cd–Mt	10K[b]	(Cd + Zn)/SH = 3 (30% SH)	pI ~ 4
Pseudomonas p.	3–7K	(Cd + Zn + Cu)/SH = 1–2	
Yeast[c]	1,3.6K	Cd/SH 2–3	
Neurospora[c]	2K	Cd/SH 3(2)	
Mushroom (Cd-mycophosphatin)	12K	no SH, 13 Cd/mole	
Rice[c]	7K		λ_{max} ~ 260 nm
Mussel[c]			
Cd	10(20)K	12–26% SH	No aromatics
Hg		8 Cd + 1 Zn/mole	
Whelk	8K	3 SH	
Oyster	10K		pI 5.9 (4.4)
Oyster	10,24K	low % SH	
Alga	10K	Cd/SH ~ 1 (?)	
Bacteria[c]	10K	18% SH, 3 metals/mole	pI ~ 4.3
Lobster	> 10K		
Crab – Cu	10K		Does not bind to DEAE cellulose
Crab[c]	10K	SK (>30%)	
Drosophila[c]			
Larval	5–6K		Sequence homology Res 1–40
Adult	2–3K		
Carp[d]	10K		
Rainbow[c]	12K		
	12K (Mt and novel CdBP)		

[a] Binding proteins for other metals (Cu,Hg) are specifically noted.
[b] 10K = 10,000 dalton.
[c] Possible simple relationship to Mt.
[d] Clear evidence of Mt of mammalian type.

mammalian metallothionein was taken up in another workshop of this conference (*26*). However, there are already several important indications that some of these binding proteins have chemical properties widely divergent from metallothionein (Table 5). In particular, mushroom-mycophosphatin, whelk CdBP, and the binding proteins from oyster and *Euglena gracilis* appear to bind Cd much less tightly than metallothionein. Stability constants of 10^7 at pH 7 were reported, which would seem to be too small to prevent a wide distribution of Cd among components of cells. This is a provocative finding, which, if supported in future studies, will require researchers to reconsider the roles of these binding proteins within the cellular milieu.

Table 5. Chemical properties of CdBP[a]

Species	
Mammalian	CdMt, $K_D \sim 10^{-15}$ (apparent dissociation constant)
Pseudomonas p.	Nonsulfur ligands from ^{113}Cd-NMR analysis
Yeast	Cd-peptide, labile sulfide present
Mushroom	Cd-mycophosphatin, half dissociated at pH 7
Whelk	CdBP, $K_D = 7 \times 10^{-6}$
Oyster	$Cd_xBP + yCd \overset{K_D}{\rightleftharpoons} Cd_{x+y}BP$, large K_D
Alga	large K_D
Crab	CuBP, Cu *donor to apohemocyanin*
Rainbow trout	

[a] A CuBP is described for crab.

Reaction of Cadmium with Ehrlich Cells

To examine the effects of Cd upon cells which contain a basal level of Zn–Mt with definable function, Ehrlich

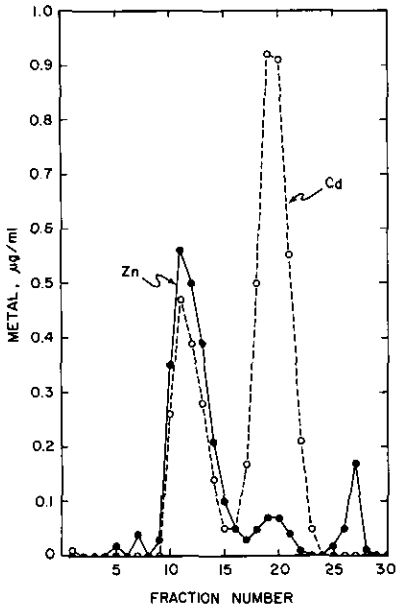

FIGURE 5. Sephadex G-75 profile of Cd and Zn in Ehrlich cell supernatant 24 hr after cells were pulsed with 170 nmole $CdCl_2$/mg cell protein for 30 min.

cells were treated with Cd intraperitoneally in mice and in culture (*27,28*). Both experiments agree that cell proliferation is halted at levels of Cd which bind in the cytosol almost exclusively to Mt. Under these conditions Mt contains both Zn and Cd. In addition, the kinetics of uptake and incorporation of thymidine into DNA in Cd-treated cells resemble those of zinc-deficient cells which have lost zinc primarily out of metallothionein (*27,29*). Thus, these results point to metallothionein as the site of action of Cd to inhibit cell proliferation.

When cells in culture are titrated with Cd and examined after 24 hr for effects and metal distribution, inhibition of cell proliferation correlates with the binding of Cd to Mt and a relative lack of non-Mt Cd (*28*). Additional Cd, which places a large amount of Cd into the non-Mt protein fraction (Fig. 5), has no further effect on cell proliferation or viability. Either the control site is Mt or it is another obscure site (CdL) for which the reaction (5) is not favorable.

$$\mathrm{Cd^*L + Cd,Zn\text{-}Mt \rightleftharpoons Cd,Cd^*\text{-}Mt + ZnL} \qquad (5)$$

Pursuing the first possibility, several ways by which the presence of Cd in Mt might disrupt the normal activities of Zn-bound to the protein are: (1) Clusters seem to act cooperatively in ligand exchange reactions. Thus, mixed metal clusters may behave differently than homogeneous metal clusters (*21,22*). (2) Change in biodegradation rate of protein and presumably metal release:

Zn-Mt in rat liver:	$t_{1/2} \sim 20$ hr
Cd,Zn-Mt:	$t_{1/2} \sim 80$ hr

(3) Change in effective apparent stability constant of cluster (*8*)

$$\mathrm{Zn_7-Mt}: \log K_{app} = 11$$
$$\mathrm{Cd_7-Mt}: \log K_{app} = 15$$

Mixed species may have intermediate K_{app}. (4) Change in kinetics of ligand substitution (*21,22*):

$$\mathrm{Zn_7-Mt} \geqslant \mathrm{Cd_7-Mt} \text{ with small ligands}$$
$$\mathrm{Zn_7-Mt} \geqslant \mathrm{Cd,Zn-Mt} \text{ with apocarbonic anhydrase}$$

It is already known that the half-time of biodegradation of Cd,Zn–Mt in rat liver is several times larger than that for excess Zn–Mt (*29*). This suggests that the mixed-metal protein differs markedly for homogenous Zn_7–Mt, despite the probable identity of their cluster structures (*31*). Indeed, the metal clusters seem to act cooperatively in metal-binding and ligand exchange reactions (*8,21,22*). Thus, mixed-metal clusters may react cooperatively and do so with different thermodynamics or kinetics than observed with either Cd_7– or Zn_7–Mt with respect to zinc bound to the protein. Studies are in progress to test these ideas.

The thrust of this workshop paper is to stress that to understand the basis of Cd-toxicity in an organism or cell, there must be a broad inquiry with the chemistry and biochemistry of Mt and other Cd-binding proteins. Indeed, in many nonmammalian systems, the binding of Cd to cellular sites besides Mt or BP seems more prominent than in mammals (Table 2). Until one defines the normal function of these various sites and how they are modified by the presence of Cd in cells, the biochemical basis of cadmium toxicity will remain elusive.

Special Problems in the Study of Nonmammalian Organisms

In addition to the biochemical considerations noted above, there are a number of other biological and nonbiological factors which are known to influence strongly cellular metal binding patterns and toxicity in nonmammalian organisms to a greater extent than observed in mammals. Normal seasonal processes such as changes in water temperature, pH, salinity, reproductive and molting cycles have all been shown to change markedly the molecular binding patterns of metals in nonmammalian species. These physiological stresses which are not encountered in mammals need to be carefully considered in evaluating expected results of metal exposure studies conducted on these organisms at different times of the year. Another biological difference that should also be considered is the fact that many nonmammalian organisms have numerous discrete developmental stages which may be differently sensitive to metal toxicity, to a greater extent than mammals. The overall point to the above is that the basic biology of nonmammalian organisms may influence metal-binding patterns and toxicity to a much greater extent than usually observed in mammals. An investigator working with metal-binding proteins in these species must hence not make assumptions about the biochemistry or biological roles of these molecules based on the mammalian literature.

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