

Mechanisms of Kidney Cell Injury from Metals

by Bruce A. Fowler¹

The most environmentally abundant toxic metals/metalloids (arsenic, cadmium, lead, and mercury) are each known to produce cell injury in the kidney but the molecular mechanisms underlying these events are now being elucidated. It is clear that the nephrotoxicity of these agents is due, in part, to the fact that urinary elimination is a major route of excretion from the body. The role(s) of molecular factors such as metal-binding proteins, inclusion bodies, and cell-specific receptorlike proteins that appear to influence renal tubule cell expression, have attracted increased interest as determinants that modulate cell populations as special risk for toxicity and renal cancer. The future of mechanistic toxicology studies with regard to how and why only certain renal cell populations become targets for toxicity from these metals/metalloids and other less common inorganic nephrotoxicants must focus on the molecular handling of these agents by target cell populations.

Introduction

The mechanisms of kidney cell injury from toxic metals are only now being understood at the molecular level due to an increased understanding of how these agents are handled by target cell populations and the roles of protective cellular mechanisms in mediating toxicity at the cellular and molecular levels of biological organization. This review examines our current state of knowledge with regard to how the most common toxic environmental metals/metalloids (lead, mercury, cadmium, and arsenic) produce renal cell injury and the roles that molecular factors such as metal-binding proteins and stress protein responses may play in these processes. Particular attention is given to the mechanisms underlying metal-induced alterations in renal gene expression and the importance that this phenomenon may have in both the cell injury process and induction of renal cancer.

In addition, the known factors that may determine why only only certain cells in certain portions of the primary functional unit of the kidney, the nephron, are susceptible to toxicity from individual toxic metals/metalloids are examined. The basic physiology of handling toxins by certain portions of the nephron is discussed from the perspective of how these transport processes may influence the molecular mechanisms of toxicity within these target cell populations. Finally, this review examines possible new classes of biological indicators of renal cell injury from toxic metallic agents because it is clear that new tests are needed to detect chronic, low-level effects from these environmentally dispersed metals/metalloids before clinical symptoms of chronic renal disease occur.

Lead

Lead is the most ubiquitous of the nephrotoxic metals, and humans are exposed to this agent in air, food, and water. Clinical studies in workers (1-5) have shown the development of renal insufficiency, after exposure to lead, and there have been several case reports of renal cancer (6-8). The mechanisms underlying these phenomena are currently not understood, but animal studies involving chronic exposure have demonstrated renal tubular damage characterized by development of pathognomonic lead intranuclear inclusion bodies (Fig. 1) and renal cancer in rodents after high-dose exposure (9). The toxic effects of lead on the kidney appear to be primarily localized in the proximal tubule (9-21). Physiological studies of lead transport in the kidney (22,23) have shown that this metal is taken up in proximal tubule cells by a process that is inhibited by tin and several other metabolic inhibitors. Brush-border membrane vesicle transport studies (24) have shown that lead is taken up by extensive membrane binding and possibly by a passive transport mechanism.

The intracellular handling of lead appears to be mediated at low dose the levels by soluble lead-binding proteins (25), which appear to act in the manner of receptors to mediate the bioavailability of Pb^{2+} to sensitive enzymes such as δ -aminolevulinic acid dehydratase (17,21,26-30) and mediate the intranuclear transport of lead and chromatin-binding (29,30) with attendant changes in renal gene expression (31). A cleavage product of α_2 microglobulin (32,33) is the principal lead binding protein (PbBp) in renal proximal tubule cells of rats (Fig. 2), whereas the human (34) and monkey PbBPs appear to have similar ion exchange characteristics and to be chemically similar, with high percentages of glutamate and aspartate amino acids (unpublished data).

The importance of these data is derived from the fact that the PbBPs appear to be a chemically similar but not identical set of proteins that bind lead at low dosages and mediate its biological

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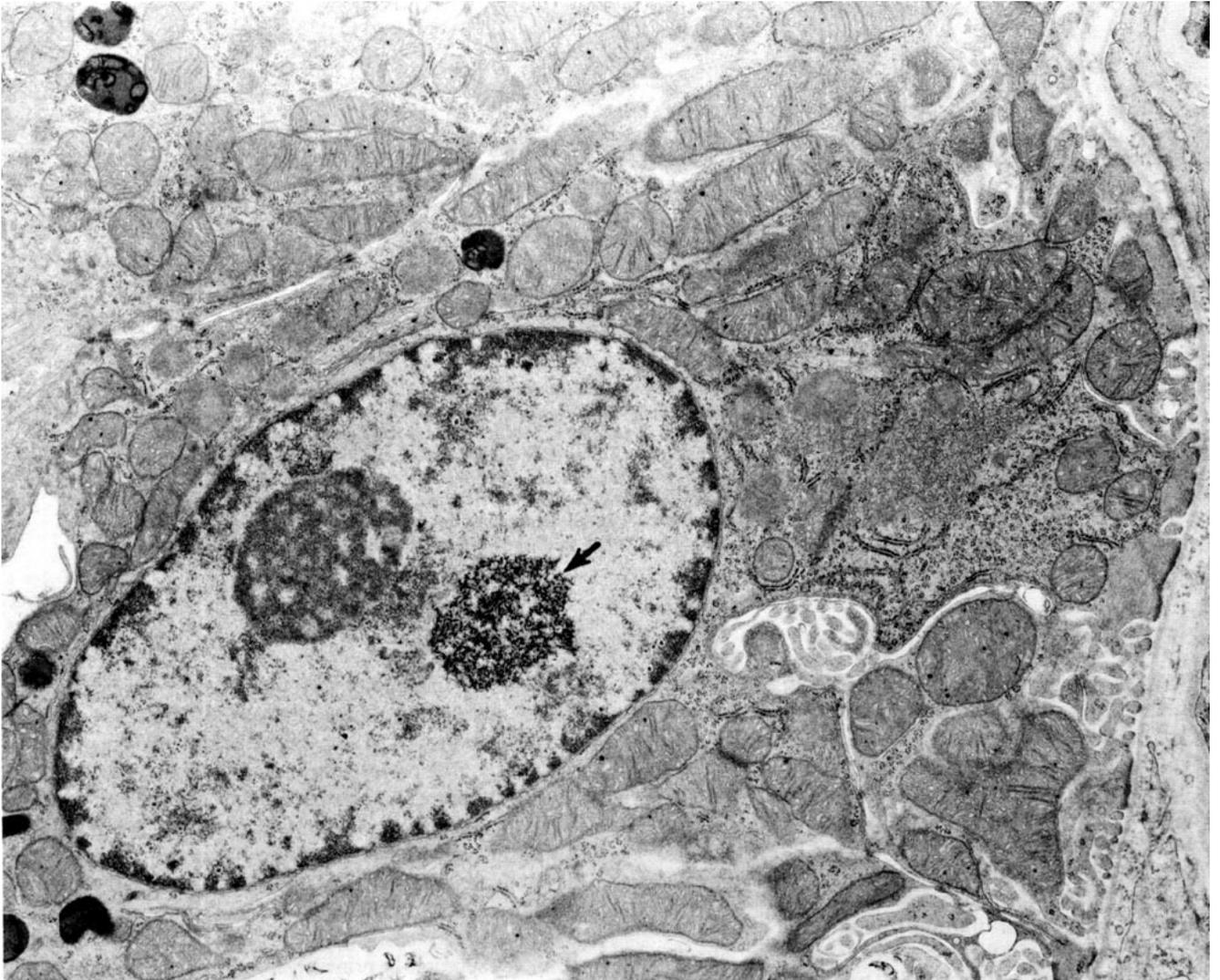


FIGURE 1. Electron micrograph of a renal proximal tubule cell showing the pathognomonic lead intranuclear inclusion body (arrow).

activity within target cell populations. It has been previously hypothesized (33,35) that the well-known lead-induced alterations in renal gene expression (30,35–38) are mediated by binding of these PbBPs to the 5' flanking regions of genes showing altered expression patterns such as outlined in Figure 2. In addition, if the reported (39) polymerization of α_2 microglobulin plays a role in the formation of the cytoplasmic lead-containing inclusion bodies (17–20), then the observed temporal relationship (31) between formation of these inclusions and coincident alterations in renal gene expression becomes even more closely linked. Further research is needed to complete ongoing molecular biology studies concerning the relationships between Pb^{2+} binding to the PbBP in rats, monkeys, and humans and altered gene expression in proximal tubule cells.

In addition, if the observed increases in excretion of the PbBP that occur in rats after low-dose lead exposure (40) also occur in

humans and monkeys, then radioimmunoassay measurement of these molecules in urine of exposed humans may prove to be a useful biological indicator of lead-induced renal damage in the future.

Organelle System Effects of Lead

Mitochondria. Renal proximal-tubule-cell mitochondria have long been known for their sensitivity to lead (9,10,17,20,21), with both morphological and biochemical alterations in structure/functional relationships. In particular, decreased respiratory function, which is linked to decreased morphological transformational capability (41), is of clear importance with regard to cell injury from this metal. Decreases in the specific activities of mitochondrial-based heme pathway enzymes have also been reported (17,21). Overall, it is clear that this organelle system is a highly sensitive, early target for lead in the kidney.

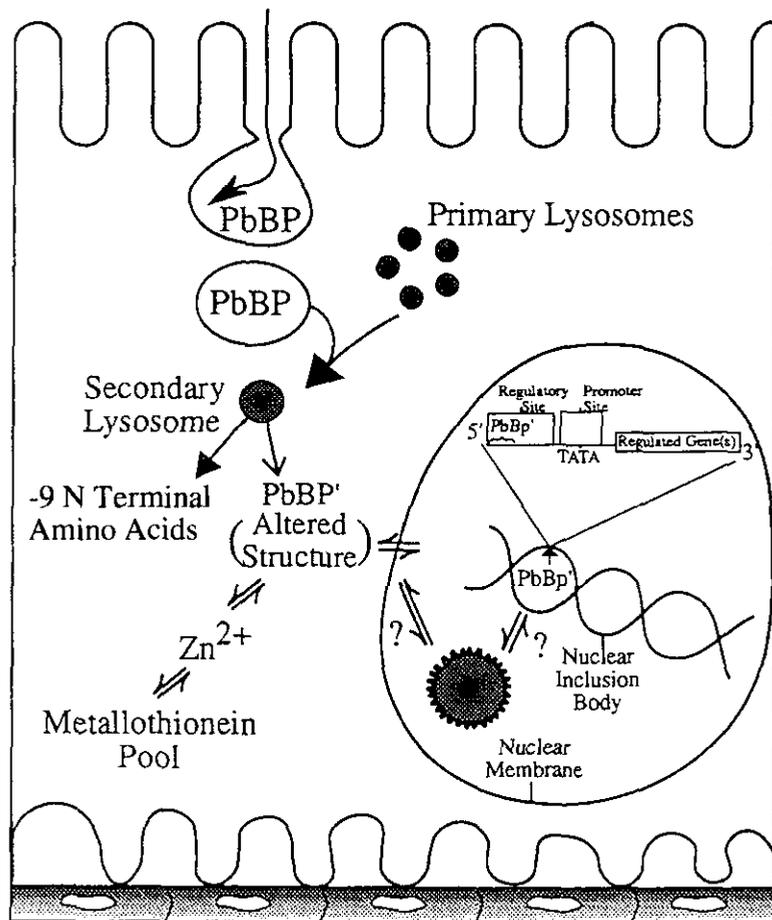


FIGURE 2. A receptor-based hypothesis for explaining how the renal lead-binding protein cleavage product of α_2 -microglobulin may interact with the renal proximal tubule cell genome to alter the gene expression changes observed in this cell type.

Nuclei. Lead-induced alterations in renal gene expression associated with formation of intranuclear inclusion bodies and tubular mitosis have been known for many years (9-20). Recent studies from a number of laboratories (31,36,42) have shown that these changes in renal gene expression and mitosis are not secondary to a cell death and replacement phenomenon. Such findings are consistent with the hypotheses discussed above regarding the receptorlike nature of the PbBPs in the kidney in mediating these effects of lead at doses below those that procedure overt cell death.

Mercury

The nephrotoxic effects of mercurials have been known for many years (43-46), and it has been clear that the anatomical site and morphological manifestations of toxicity are highly dependent on the chemical form of the mercurial involved. In general, inorganic mercury such as mercuric chloride produces effects (43-45) on the third segment (S3) of the proximal tubule, whereas organomercurials such as methylmercury tend to produce effects on the second (S2) and third (S3) segments, depend-

ing on the sex of the animal (48-51), dose and duration of exposure and exposure to agents such as dieldrin (48) and phenobarbital (50). Agents such as dieldrin and phenobarbital, appear to induce oxidative demethylation in the S3 segment of the proximal tubule and increase the excretion of inorganic mercury from the kidney (50). The nephrotoxicity of mercurials is thus dependent on a number of factors that may mediate which cells in the nephron are affected and presumably the mechanism(s) of toxicity at the organelle and molecular levels of biological organization. The organelle effects of mercurials are discussed below.

Cell Membranes

Studies from a number of laboratories (52,53) have shown that acute injection of mercuric chloride produces a number of effects on renal proximal tubule cells of the S3 segment. Marked blebbing and exfoliation of the brush-border membranes is among the earliest of these phenomena and undoubtedly plays a role in the massive influx of calcium associated with cell death after exposure to mercuric chloride. This phenomena is not usually observed with exposure to methylmercury.

Mitochondria

Both organic and inorganic mercury exposure have been shown to both damage renal proximal tubule cell mitochondria (54-59), with loss of respiratory function and alterations in key enzymes in the heme biosynthetic pathway (54). These decreases in heme biosynthetic pathway enzymes are associated with development of a characteristic porphyrinuria pattern (54), which appears to be of renal origin. It is clear that such decreases in these essential biochemical processes may play an important but as yet incompletely delineated role in renal cell injury from these agents.

Lysosomes

Exposure to both inorganic and organic mercurials (51) has been shown to alter the structure and function of the renal-tubule-cell lysosome apparatus. This structural and biochemical perturbation of the renal lysosomal system by mercurials may result in the development of proteinuria. The release of the metal-binding protein metallothionein, which binds mercury but not methylmercury into the urine, has been proposed as a possible biological monitoring test for exposure of persons to mercury vapor.

Cadmium

In recent years, there has been intensive research into the mechanisms underlying the well-documented proteinuria (61,22) and calcuria (62) observed in persons after chronic elevated exposure to cadmium. The metal-binding protein metallothionein (MT) has been shown by workers in a number of laboratories to play several central roles in the mechanisms underlying these phenomena. There have been a number recent reviews on this protein with regard to its chemistry and biochemistry (63,64) but briefly, cadmium metallothionein (cdMT) synthesized in the diver in response to cadmium exposure is released into the circulation and transported to the kidney, where it is reabsorbed with great efficiency (66,67) and rapidly degraded (66-70), with the release of Cd^{2+} ions that stimulate the synthesis of MT within proximal tubule cells of the S1 and S2 segments (67-70). This process continues until the finite capacity of the cells is exceeded either as a function of chronic exposure or dose. Once this threshold is exceeded, renal cell injury with concomitant proteinuria and calcuria is initiated. The mechanisms of Cd^{2+} -induced renal tubule cell injury are described below.

Lysosome System

The uptake and degradation of CdMT in the renal proximal-tubule cells is associated with the initial increased presence of cadmium in the lysosomal fractions (67), followed over a period of several hours by the increased presence of cadmium in the cytosol, where it is bound to both renal MT and a non-MT fraction. With the passage of time, the percentage of Cd^{2+} bound to the MT increases and that of the non-MT fraction decreases. Studies from this laboratory have shown that it is the Cd^{2+} associated with the non-MT fraction that is temporally associated with cytotoxicity (69,70). The ultrastructural appearance of cells exposed to parenteral doses of CdMT shows a characteristic

pattern of vesiculation and an increased number of electron-dense lysosomes (Fig. 3), and this is temporally associated with decreases in lysosomal protease activity (70), low molecular weight proteinuria (69,70), and calcuria (72-75). These findings are similar to those noted in persons exposed to cadmium for prolonged time periods (62) as noted above. The interpretation of these findings is that Cd^{2+} in the non-MT fraction is capable of interfering with the normal process of lysosomal biogenesis, which results in the decreased reabsorption and degradation of low molecular weight proteins from the urinary filtrate, thus resulting in a tubular proteinuria similar to that observed in persons exposed to cadmium for long time periods. At present, the mechanism of the calcuria is unclear. Some data (72) indicate that most of the calcium in the urine is nonsionized and probably protein bounds suggesting that it is secondary to the proteinuria, whereas more recent studies using shorter collection times (75) have shown that the increased excretion of calcium in the urine occurs before the main increases in protein excretion. Further research is needed to clarify the relationships between the proteinuria and calcuria.

Calmodulin

To elucidate the mechanisms of cadmium-induced renal cell injury, recent preliminary *in vitro* studies (76,77) using CdMT have focused on relationships between morphological alterations in proximal tubule cells in culture, changes in intracellular Ca^{2+} concentrations, and induction of stress proteins. The results to date indicate that induction of stress proteins followed by the cellular vesiculation phenomenon shown in Figure 3 above occur well before measurable alterations in intracellular Ca^{2+} , which occur only as the cells begin to die. Such data are important because they suggest that the observed toxic phenomena are not secondary to altered calcium-induced mechanisms but rather a function of Cd^{2+} binding to effector molecules early in the toxic process. A primary candidate for this role is calmodulin because *in vitro* studies from a number of laboratories (78-81) have shown that Cd^{2+} is capable of activating this protein. Activation of calmodulin by the Cd^{2+} ions not bound to MT could damage the cytoskeleton, which is thought to play an important role in the process of lysosomal biogenesis (82). Studies are currently in progress to evaluate this hypothesis with regard to competition between MT and calmodulin for the Cd^{2+} ions and to elucidate the role(s) of stress proteins in mediating Cd^{2+} induced cell injury.

Arsenic

There are relatively few clinical reports of arsenic-induced kidney cell injury in comparison with those of lead, mercury, and cadmium, but such cases have been documented (83). In comparison with the other metals discussed above, the chemical form and oxidation state of the arsenical of concern is of even more importance to understanding the mechanisms of cell injury. Inorganic pentavalent arsenic (As^{5+}) and trivalent arsenic (As^{3+}) vary markedly in their acute toxicity and mechanisms of biological action. Methylation of these inorganic forms to methylarsonic acid and dimethylarsinic acid in the liver further complicate an understanding of *in vivo* renal toxicity because they are excreted by the kidney in the urine.

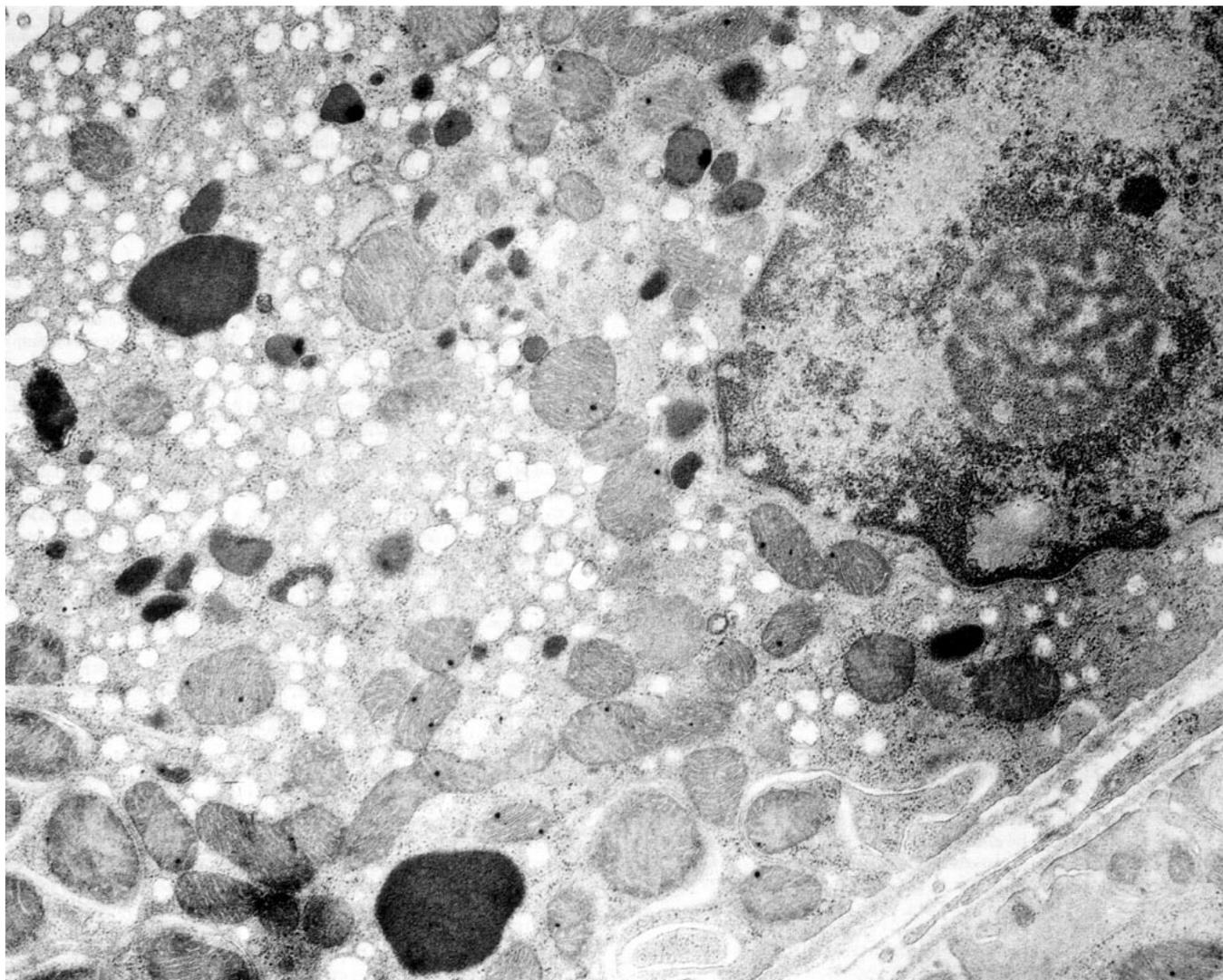


FIGURE 3. An electron micrograph of a renal proximal-tubule cell from an animal given a single IP injection of cadmium metallothionein 8 hr earlier, showing characteristic vesiculation pattern and small, dense lysosomes.

Furthermore, early studies by Ginsburg (84,85) showed arsenate (As^{5+}) is actively transported by the kidney tubules and that a small fraction of this form is reduced to As^{3+} , which is the more acutely toxic chemical form. These types of data greatly complicate any understanding of the mechanisms of arsenical-induced neurotoxicity because metabolic interconversion of these arsenicals by the renal proximal tubule cells would yield both species present.

At the organelle of biological organization, the mitochondrion is a major target site of action for all inorganic arsenicals (86). Combined ultrastructural/biochemical studies (87) conducted on kidneys of rats exposed to arsenate (As^{5+}) in drinking water for prolonged periods of time showed *in situ* swelling associated with decreased respiratory function. More recent studies (88) have shown that exposure of animals to arsenic causes induction of several stress proteins in the kidney, indicating that the genetic machinery in the nuclei is also being affected by arsenical ex-

posure. It is not clear whether this phenomenon is a primary response to arsenic entering the nucleus or secondary to other aspects of the cell injury process such as decreased energy production. In any event, further research is needed to understand how arsenicals are able to produce damage to proximal-tubule cell structure and function.

Metal-Metal Interactions

It should be noted that the above discussion has considered mechanisms of metal-induced renal cell injury as if exposure occurred to only one metal at a time. In reality, it is clear that environmental exposure to metals involves all of these agents at the same time and in varying concentrations from different media. To be truly useful, the biological indicators described above must be able to provide information in a such an exposure matrix. Previous studies (89,90) using a lead-cadmium-arsenic

exposure design have shown that combinations of these metals/metalloids produce unique porphyrinuria patterns and that cadmium exposure has a marked effect on total renal uptake of lead and the formation of inclusion bodies. More recent studies (29,30) on the renal PbBP have shown that cadmium is the most effective competitor for displacing lead from these molecules, thus providing a mechanism for these *in vivo* observations. The data also suggest that a molecular competition must exist between the renal PbBP and metallothionein for metals such as zinc and cadmium. The other interesting metal-metalloid interaction is that known for mercury and selenium, with formation of unique crystalloid intranuclear inclusion bodies (47). Formation of these structures appears to attenuate the nephrotoxicity of Hg²⁺ in the kidney. The importance of the above discussion is that it indicates the need to consider interactions between metals in mechanism-based risk assessment situations where more than one element is present.

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