Understanding Renal Toxicity of Heavy Metals*

GARY L. DIAMOND1 AND RUDOLFS K. ZALUPS2

1Syracuse Research Corporation, 6225 Running Ridge Road, North Syracuse, New York 13212-2510, and 2Division of Basic Medical Science, Mercer University School of Medicine, Macon, Georgia 31207

ABSTRACT

The mechanisms by which metals induce renal injury are, in general, poorly understood. Characteristic features of metal nephrotoxicity are lesions that tend to predominate in specific regions of the nephron within specific cell types. This suggests that certain regions of the nephron are selectively sensitive to specific metals. Regional variability in sensitivity could result from the localization of molecular targets in certain cell populations and/or the localization of transport and binding ligands that deliver metals to targets within the nephron. Significant progress has been made in identifying various extracellular, membrane, and intracellular ligands that are important in the expression of the nephrotoxicity of metals. As an example, mercuric chloride induces a nephropathy that, at the lowest effective doses, is restricted primarily to the S3 segment of the proximal tubule, with involvement of the S2 and S1 segments at higher doses. This specificity appears to be derived, at least in part, from the distribution of enzymes and transport proteins important for the uptake of mercury into proximal tubule cells: apical \( \gamma \)-glutamyltranspeptidase and the basolateral organic anion transport system. Regional distributions of transport mechanisms for binding proteins appear to be important in the expression of nephrotoxicity of metals. These and other new research developments are reviewed.

Keywords. Albumin; cadmium; cysteine; glutathione; kidney; mercury; metallothionein; nephrotoxicity; organic anion; \( p \)-aminohippurate; transport; uranium

INTRODUCTION

Characteristic features of metal nephrotoxicity are lesions that tend to predominate in specific regions of the proximal tubule and in the glomerulus. This suggests that these regions of the nephron may be selectively sensitive to specific metals. Regional variability in sensitivity to metals could result from the localization of transport and binding ligands that deliver metals to targets within the nephron and/or the localization of molecular targets in certain cell populations. Although significant progress has been made in identifying various extracellular, membrane, and intracellular ligands that are important in the expression of the nephrotoxicity of metals, the mechanisms by which metals induce renal injury remain poorly understood. This is due, at least in part, to the complexity of the ligand interactions that dominate the disposition of metals in living organisms. For the most part, metals affect molecular processes through binding interactions with molecular targets, generally nucleophiles such as proteins and nucleic acids. These interactions may alter the normal function of the target molecule by displacing essential elements from the molecule, altering its structure or changing its rate of metabolism or its interactions with other important ligands. While it is possible to study in great detail the binding interactions of metals and the consequences of binding on the function of specific binding ligands, it is extremely difficult to determine which of these interactions contribute to the biochemical changes that give rise to toxicity. Thus, elucidating mechanisms of toxicity requires an understanding of the pathophysiology that underlies toxicity, within which, specific molecular interactions can be placed in appropriate context.

GENERAL FEATURES OF METAL NEPHROTOXICITY

The pathophysiology of acute uranium nephrotoxicity has been extensively examined and reviewed (26) and exemplifies some general features of metal nephrotoxicity. Historically, intense interest in the toxicology of uranium originated with the Manhattan Project and the atomic energy program that required the processing and handling of large amounts of hexavalent uranium compounds (\( U^{VI} \)) and that presented significant occupational health challenges (46). As new techniques for studying kidney morphology and function developed, research on uranium nephrotoxicology continued with the focus of using hexavalent uranium compounds (e.g., uranyl acetate, uranyl fluoride, and uranyl nitrate) to explore mechanisms of acute renal failure (33). The results of these studies describe the sequence of events that can occur in response to an acutely toxic dose of uranium and, although not entirely applicable to other metals, illustrate important kinetic and functional characteristics of proximal tubular and glomerular injury that are relevant to other metals.

Functional and morphologic changes that follow a single parenteral dose of uranyl nitrate, 6–15 mg U kg body weight (BW), in rats are summarized in Figs. 1 and 2. A qualitatively similar sequence of changes has been established for dogs and rabbits exposed to uranyl acetate (56, 91) and, in less detail, for dogs and rats exposed to uranyl fluoride (26, 63, 64, 74). Principal features of this sequence can be divided into glomerular effects, including perfusion and filtration defects and morphological abnormalities, and tubular effects, including transport and permeability defects and necrosis. In general, a discussion of mechanisms of metal nephrotoxicity can be similarly
Mechanisms of Glomerular Toxicity

At sufficiently high dose levels, most heavy metals will depress the glomerular filtration rate (GFR). The Starling equation for capillary filtration, which describes the major determinants of GFR, provides a conceptual model with which to explore potential mechanisms:

\[ J_v = K_f [(P_{gc} - P_i) - (\pi_{gc} - \pi_i)] \]

\[ K_f = L_p A_f \]

- \( J_v \) = single nephron GFR (nl min\(^{-1}\))
- \( K_f \) = glomerular ultrafiltration coefficient (nl min\(^{-1}\) mmHg\(^{-1}\));
- \( P_{gc} \) = glomerular capillary hydraulic pressure (mmHg);
- \( P_i \) = tubular fluid hydraulic pressure (mmHg);
- \( \pi_{gc} \) = glomerular capillary fluid oncotic pressure (mmHg);
- \( \pi_i \) = tubular fluid oncotic pressure (mmHg);
- \( L_p \) = hydraulic conductivity of the glomerular filter (nl min\(^{-1}\) cm\(^{-2}\) mmHg);
- \( A_f \) = filtration surface area (cm\(^2\)).

Net filtration pressure is determined by the balance between hydraulic \((P_{gc} - P_i)\) and oncotic \((\pi_{gc} - \pi_i)\) pressure gradients along the glomerular capillary. Filtration proceeds along the length of the capillary as long as net hydraulic pressure exceeds net oncotic pressure. If at some point along the glomerular capillary net oncotic pressure increases sufficiently to equal net hydraulic pressure, then a condition of filtration pressure equilibrium is established and filtration ceases at that point.

A consistently reported feature of uranium-induced acute renal failure in dogs, rabbits and rats is a decrease in renal outer cortical blood flow and glomerular perfusion (34, 35, 77, 90, 91). Decreased glomerular perfusion could lower GFR by decreasing glomerular capillary hydraulic pressure \((P_{gc})\) or by shifting the point of filtration pressure equilibrium toward the afferent end of the glomerular capillary, thereby decreasing the length of the capillary that is available for filtration. In at least one study, GFR was observed to decrease in the absence of significant changes in renal plasma flow in rats treated with uranyl nitrate (12). Therefore, decreased renal plasma flow does not appear to be required for decreasing GFR, and other factors must be involved.

A rise in tubular hydraulic pressure \((P_i)\), as a result of impaired tubular solute and fluid reabsorption or tubular blockage, also may contribute to decreased GFR. Although elevated tubular hydraulic pressure has been reported to occur in rats exposed to 6 mg U/kg BW parenteral uranyl nitrate (72) and in isolated perfused dog kidneys exposed in vitro to uranyl nitrate (76), it is not a consistent finding (34, 90); thus, it cannot be the sole mechanism responsible for decreasing GFR.

A complete quantitative description of the Starling forces in the glomerulus is possible in the Munich-Wistar rat, which possesses glomeruli that are located on the surface of the kidney and can be micropunctured for measurement of glomerular hydrostatic and oncotic pressures (14). In this animal model, a 50% decrease in single nephron filtration rate was observed during the first 6 hr after injection of 15 mg U/kg BW uranyl nitrate, concurrent with significant elevation of net glomerular filtration pressure and in the absence of a change in renal plasma flow (12). This study demonstrates the importance of changes in the ultrafiltration coefficient \((K_f)\) in uranium-induced depression of GFR.

Abnormalities in the ultrastructure of the glomerulus occur concurrently with the decrease in GFR. In rats, diameter and density of glomerular endothelial cell fenestrae decrease 7 hr after a parenteral dose of 9–15 mg U/kg BW uranyl nitrate (6). Such changes may give rise to a decrease in filtration surface area \((A_f)\) and, thereby, a decrease in ultrafiltration coefficient. Endothelial changes are followed by distortions of the glomerular visceral epithelium (6, 43), suggesting that the endothelium, rather than the epithelium, may be the primary target of uranium-induced depression of GFR. In rabbits, concurrent endothelial and epithelial changes in the glomerulus have been reported (56). Epithelial changes include spreading and flattening of the visceral epithelial cells (podocytes) and loss of podocyte processes, leading to decreased epithelial coverage of the glomerular endothelium.

Fig. 1.—Primary sites of uranium-induced injury in the rat (stippled areas). Necrosis of the pars recta segment of the proximal tubule, and to a lesser extent, distal segments of the nephron are prominent features. Ultrastructural abnormalities in the glomerular endothelium and epithelium have been documented at dose levels exceeding 6 mg U/kg body weight.
Both uranium and mercury can induce albuminuria in animals (27, 68, 116). The albuminuria may reflect impaired reabsorption of protein, as it occurs in association with cellular and tubular necrosis; however, increased filtration of albumin also may be a contributing factor. Effacement of glomerular epithelial cells would be expected to alter the sieving properties of the glomerulus and may contribute to glomerular proteinuria; however, definitive evidence for increased filtration of albumin in animals treated with uranium has not been established. In rabbits, clearance of neutral dextrans having molecular weights of 70,000-90,000 daltons is not affected by a parenteral dose of 0.2 mg/kg BW uranyl acetate, suggesting that mechanisms other than a change in the size selectivity of the glomerular filter contribute to proteinuria (36). A rigorous examination of the effect of uranium compounds on the charge selectivity of the glomerulus has not been reported.

Our understanding of mercury-induced albuminuria is no more complete than that for uranium; that is, it is unclear to what extent albuminuria is of glomerular or tubular origin. Evidence for mercury-induced glomerular albuminuria is indirect. In rabbits, rats, and mice, multiple exposures to inorganic mercury induce the production of antibodies against the glomerular basement membrane, deposition of immune complexes in the mesangium and glomerular basement membrane, and glomerular nephritis (11, 29-31, 48, 83, 86, 96). In general, immune-complex glomerulonephritis has been associated with a disruption of the charge and/or size-selective sieving properties of the glomerulus and increased filtration of albumin and other plasma proteins (2, 13, 99); however, this has not been rigorously explored in animals treated with mercury.

**MECHANISMS OF TUBULAR TOXICITY**

In general, nephrotoxic heavy metals characteristically produce lesions in the proximal tubule. These lesions often show a degree of dose–response heterogeneity across the length of the proximal tubule. For example, chronic exposure to cadmium chloride or acute injections of potassium dichromate characteristically produce tubular necrosis that initiates at the lowest effective dose levels in the pars convoluta of the proximal tubule and then progresses to more distal regions of the tubule. By contrast, mercuric chloride (119), uranyl compounds (43), and platinum compounds (28) produce a necrosis that originates at the lowest effective dose levels in the pars recta of the proximal tubule. Uanyl compounds exemplify the extreme of this dose–response heterogeneity. In rats, morphological changes in the pars recta segments of the proximal tubule, evident within 6 hr after a 6-mg U/kg dose of uranyl nitrate, include focal loss of periodic acid-Schiff (PAS)-positive staining and loss of brush border from the proximal tubule (34, 43). The injury progresses in severity for the first 5 days, culminating in near complete necrosis of the pars recta segment of the proximal tubule with the pars convoluta segment remaining relatively free from injury (43). Distortion, focal necrosis, and casts in the thick ascending limb of the loop of Henle, distal tubule and collecting tubule follow proximal
tubule injury (27, 43). Regeneration of the injury with squamous epithelium begins within 1 wk after dosing, during the time of peak severity of injury; however, atropic tubules, casts and interstitial fibrosis have been reported 56 days after 6 mg U/kg BW uranyl nitrate, suggesting a chronic aspect of the injury (44). After lower doses of uranyl fluoride, 0.7–1.3 mg U/kg BW, restoration of the normal morphology of the tubule is completed within 35 days after dosing (27).

The mechanisms by which uranium and other metals produce localized damage to specific regions of the proximal tubule are not understood and remain one of the most significant gaps in our understanding of metal nephrotoxicology. Regional sensitivity of the proximal tubule appears to be preserved in some in vitro preparations of kidney slices. Ruegg et al (85) examined histological changes that occurred when renal cortical slices of rat kidney were incubated with mercuric chloride or potassium dichromate. Consistent with the in vivo response with these 2 chemicals, the pars recta region was more sensitive to mercuric chloride while the pars convoluta region was more sensitive to potassium dichromate. Thus, the regional differences in sensitivity seem to be a characteristic of the renal tubular epithelium and are not the result of hemodynamic or neurogenic factors.

An understanding of the mechanism for dose–response heterogeneity in the proximal tubule will come with identification of the various extracellular, membrane, and intracellular ligands that are important in the expression of the nephrotoxicity. Significant recent progress has been made on this problem as it relates to the nephrotoxicology of inorganic mercuric mercury (119, 120). What follows is a summary of our current understanding of this subject, and, where relevant, other heavy metals are discussed.

**Relationship between Sites of Mercury Accumulation in the Kidney and Toxicity**

Acute doses of inorganic mercuric mercury (Hg2+) induce cellular necrosis in the proximal tubule. The injury is most prominent in the pars recta (S2 and S3 segments) at low doses, with involvement of the pars convoluta of the proximal tubule and distal segments of the nephron at higher doses (73, 102, 119). The location of the injury along the nephron gives rise to a characteristic histological presentation typified by cellular necrosis that is most severe in the inner cortex and outer stripe of the outer medulla (OSOM). Functional correlates of mercury-induced cellular degeneration include loss of enzymes in the luminal brush-border membrane of the proximal tubule that can be detected as an increase in the excretion of brush-border enzymes in urine (e.g., alkaline phosphatase, γ-glutamyltranspeptidase [γGT]). With more advanced cellular and tubular necrosis, intracellular enzymes (e.g., lactate dehydrogenase, N-β-D-glucosaminidase) are excreted in urine and impaired reabsorption of water and solutes in the proximal tubule gives rise to diuresis, glucosuria, amino aciduria, and proteinuria (73, 115, 116).

The pattern of histological changes in the renal cortex and outer medulla agrees well with the observed pattern of accumulation of inorganic mercury that occurs predominantly in the same regions of the kidney (9, 94, 123). Autoradiographic and histochemical techniques have further localized the sites of inorganic mercury accumulation to the proximal tubule (82, 94). These observations suggest that injury results from a direct interaction between mercury and the proximal tubule. Analysis of the location of mercury along the proximal tubule has been attempted to define further the relationship between the site of accumulation and the site of injury. In the BALB/c mouse, after administration of a nephrotoxic dose of mercuric chloride, and in the Sprague-Dawley rat, after administration of either a nontoxic or nephrotoxic dose of mercuric chloride, mercury was localized with autometallographic techniques to the S1, S2, and S3 segments with more abundant deposits in the pars recta, S2, and S3 segments (47, 49, 103). Unilateral nephrectomy and subsequent compensatory renal growth, which increases the severity of mercury-induced injury to the pars recta of the proximal tubule and increases accumulation of mercury in the OSOM in the rat, also increase the abundance of autometallographically detected mercury in the S2 and S3 segments of the proximal tubule (103).

These observations suggest that injury to the pars recta segment of the proximal tubule may be directly related to the association of mercury with the pars recta. In the rabbit, after administration of a nontoxic dose of mercury, the mercury contents (per unit length) of microdissected S1 and S2 segments of proximal tubule were similar and approximately twice that of the S3 segment (107). Thus, although the pattern of injury along the proximal tubule is consistent with the overall pattern of abundant accumulation of mercury in the pars recta, mercury is not exclusively accumulated in this segment of the proximal tubule. Variables other than the total accumulation of mercury must contribute to the pronounced vulnerability of the pars recta segment of the proximal tubule to mercury.

A more severe injury to the renal proximal pars recta, relative to the pars convoluta, has been observed in rat renal cortical slices incubated in vitro with 100 μM mercuric chloride (85), suggesting that the mechanisms responsible for the pronounced sensitivity of the pars recta segment may not require glomerular filtration or other hemodynamic factors present in vivo. In contrast to the slice, when mercuric chloride (18 μM) was perfused directly into isolated rabbit proximal tubular segments, toxicity occurred in S1, S2, and S3 segments without evidence for selective or more severe toxicity to the S2 or S3 segments (8, 124). Three hypotheses can be derived from these observations: (a) 18 μM mercuric chloride, presented to the luminal side of the proximal tubule epithelium, is above the threshold for toxicity of the S1, S2, and S3 segments of the proximal tubule, (b) selective toxicity to the S2 and S3 derives from the delivery of mercury to the basolateral side of the proximal tubule; or (c) selective toxicity requires a level of structural or metabolic integrity of the renal cortex and outer medulla that is preserved in the slice but not in the isolated perfused tubule.
Model of Transport of Mercury at the Luminal Membrane

Fig. 3.—Hypothetical model of acivicin-sensitive transport of mercury at the luminal membrane of the renal proximal tubule. A complex between Hg$^{2+}$ and GSH (GSH-Hg-GSH) is filtered at the glomerulus and catabolized by the enzymes γGT and dipeptidases located on the luminal membrane. The resulting mercury-cysteine complex (Cys-Hg-Cys) is transported across the luminal membrane via a carrier. Cysteine has been shown to enhance the uptake of Hg$^{2+}$ by luminal membrane vesicles isolated from rat kidney. The nature of a Cys-Hg-Cys carrier has not been elucidated but is depicted as a sodium (Na$^+$)-coupled mechanism. A dipeptide carrier (7) also may transport a dipeptide complex with Hg$^{2+}$ (GlyCys-Hg-CysGly), or the GSH-Hg-GSH complex could be a transport substrate.

Transport Mechanisms for Low-molecular-weight Mercury Complexes

Although neither of the above hypotheses can be eliminated at present, there is evidence for uptake of mercury into the proximal tubule from both the luminal and basolateral side of the tubule (Figs. 3 and 4). Glomerular filtration can be prevented and/or reduced to negligible values by ureteral ligation during a mannitol diuresis (71). In rats, this "stop-flow" procedure decreases uptake of an injected dose of mercuric chloride into the renal cortex and OSOM by approximately 40–50%, suggesting that approximately half of the mercury uptake occurs.

Model of Transport of Mercury at the Basolateral Membrane

Fig. 4.—Hypothetical model of PAH-sensitive transport of mercury at the basolateral membrane of the renal proximal tubule. A Hg$^{2+}$ complex (R$^-$–Hg–R$^-$), possibly Cys–Hg–Cys or GSH–Hg–GSH, is transported across the basolateral membrane by a sodium-coupled mechanism that is inhibited by PAH and probenecid. Sodium-coupled, probenecid-sensitive transport of GSH has been demonstrated in basolateral membrane vesicles isolated from rat kidney (59). Alternatively, PAH and probenecid sensitivity also may result from coupling of the transport of a Hg$^{2+}$ complex via an anion-exchange carrier on the basolateral membrane that is inhibited by PAH or probenecid.
from the glomerular filtrate and, therefore, from the luminal side of the tubule, and half of the uptake occurs at the basolateral side of the tubule (123). Most of this "basolateral component" of mercury uptake, approximately 75–80%, can be inhibited by an intravenous injection of the organic anion p-aminohippurate (PAH) given prior to mercuric chloride during stop-flow conditions; suggesting the involvement of an organic anion transport mechanism in the basolateral uptake of mercury (123). The enhanced uptake of mercury into the OSOM that occurs in rats that have undergone unilateral nephrectomy and compensatory renal growth is completely abolished under stop-flow conditions and uptake is further decreased by a combination of stop-flow and pretreatment with PAH (106). Therefore, unilateral nephrectomy and compensatory renal growth appear to enhance, predominantly, a luminal uptake mechanism for mercury.

Evidence has been provided for at least 3 mechanisms for the uptake of mercury into the proximal tubule epithelium, although numerous other mechanisms may exist. An organic anion-sensitive mechanism, previously eluded to, appears to be involved in the uptake of mercury from the basolateral side of the rat proximal tubule (123). An acivicin-sensitive mechanism has been shown to exist in the mouse kidney (92, 93) and rat kidney (10, 25, 105). Acivicin is an inhibitor of the enzyme γGT which cleaves the γ-glutamyl moiety from glutathione (GSH). The enzyme is located in the kidney, predominantly in the pars recta segment of the proximal tubule (45), and predominantly in the luminal membrane, although the enzyme also has been associated with the basolateral membrane and renal microvasculature (24, 88). In rats, after an injection of a nontoxic dose of mercuric chloride (0.5 μmol/kg), near complete inhibition of renal γGT activity by acivicin decreases uptake of mercury in the renal cortex by approximately 65%. The acivicin-induced decrease in mercury uptake occurs almost exclusively in the renal cortex, whereas PAH inhibits uptake of mercury in both the cortex and outer medulla (105). Treatment of rats with acivicin and PAH in combination inhibits mercury uptake in the renal cortex by approximately 90% and in the outer medulla by approximately 50% (105). Furthermore, ureteral ligation abolishes the effect of acivicin on mercury uptake, suggesting that the acivicin-sensitive mechanisms resides predominantly, if not exclusively, on the luminal side of the proximal tubule. Thus, the acivicin-sensitive and PAH-sensitive mechanisms appear to be distinct mechanisms and, together, account for most (possibly as much as 80%) of the mercury uptake in the proximal tubule.

It is possible that the PAH-sensitive and acivicin-sensitive mechanisms involve some common transport substrates. Hg²⁺ readily forms highly stable S–Hg complexes with thiols such as cysteine and GSH (38, 81), and such complexes would be expected to form in plasma given that the concentrations of reduced cysteine and GSH in plasma are approximately 10 μM (60). Furthermore, the catabolism of GSH to its constituent amino acids by γGT and dipeptidases in the brush-border membrane of the proximal tubule would be expected to give rise to Hg–cysteine in the tubular lumen. Studies of basolateral membrane vesicles isolated from rat kidney cortex and primary cultures of rat proximal tubular cells have revealed PAH- and/or probenecid-sensitive transport mechanisms for GSH, S–GSH conjugates, and S–cysteine conjugates (57–59). Thus, it is conceivable that Hg–cysteine and Hg–GSH complexes may be substrates for a PAH-sensitive transport mechanism in the proximal tubule, although evidence for this is currently lacking. Both GSH and cysteine, when administered with mercuric chloride, increase the uptake of mercury in the renal cortex and outer medulla (108, 109). Coadministration of cysteine increases the severity of nephrotoxicity of mercuric chloride (110). Studies of the transport of Hg–cysteine and Hg–GSH complexes have been attempted with preparations of luminal and basolateral membrane vesicles isolated from rat kidney; however, rapid and extensive binding of mercury to the membranes severely limits the quantification of mercury transport fluxes in this preparation. Therefore, an effect of cysteine or GSH on the amount of mercury taken up by the membranes could represent an effect on binding and/or transport because binding could occur before, during, or after transport has occurred. Cysteine in a 3- or 10-fold excess with Hg²⁺ increased mercury uptake by the luminal membrane vesicles, relative to that which occurred with Hg²⁺ alone (121, 122). GSH, on the other hand, decreased mercury uptake in both the luminal and basolateral membrane vesicles. The effect of cysteine on mercury uptake may reflect a direct or indirect coupling of Hg²⁺ and cysteine fluxes into or across the luminal membrane vesicle or, possibly, a ligand exchange between cysteine and sulfhydryls at the membrane surface. It also is conceivable that a dipeptide carrier on the luminal membrane (7) may transport a dipeptide complex with Hg²⁺ (GlyCys–Hg–Cys–Gly). Further study is needed to sort out these possible mechanisms.

Transport of Mercury–Protein Complexes

A third possible mechanism of uptake mercury in the proximal tubule is the endocytosis of Hg²⁺ bound to filtered serum albumin. Mercuric mercury in plasma is bound extensively to albumin (17), the most abundant (approximately 1 mEq) sulfhydryl in plasma (15, 51). The filtration fraction of albumin in the rat is approximately 0.0004 (79, 80); therefore, a small fraction of the Hg–albumin in plasma would be expected to enter the lumen of the early proximal tubule in the glomerular filtrate. Crude estimates of the relative magnitudes of filtered Hg–albumin and filtered low molecular weight mercury complexes (Hg–LMW) can be made by assuming the following: 50% of the mercury in blood is in the plasma fraction (104), of which 98% is bound to albumin (17) and the remaining 2% consists of Hg–LMW (e.g., Hg–GSH), a GFR of 1 ml·min⁻¹ per kidney in the rat, and a filtration fraction of 0.0004 for Hg–albumin complex and 1.0 for Hg–LMW. The above assumptions yield a plasma clearance of Hg–albumin due to glomerular filtration that is approximately 2% that of filtered Hg+LMW. Filtered albumin is extracted from the tubular fluid in the proximal tubule through the process of endocytosis and transferred to lysosomes for catabolism (67). A similar mechanism...
could result in the endocytosis of filtered Hg–albumin. Proteinuric rats given an injection of a nontoxic dose of mercuric chloride excrete a substantially larger fraction of urinary mercury as Hg–albumin compared to normal nonproteinuric rats and have a larger fraction of total cellular mercury associated with the lysosomal fraction (68, 70). These observations suggest that Hg–albumin may be taken up from the lumen of the proximal tubule, possibly by endocytosis. Although it has been reported that albumin may increase the severity of toxicity of mercuric chloride in the isolated perfused rabbit proximal tubule (124), more recent studies suggest that albumin substantially decreases the uptake and toxicity of mercury in this preparation (R. K. Zalups, unpublished observations). Mercury can induce proteinuria in animals and, in rats, mercuric chloride-induced proteinuria is associated with increased excretion of Hg–albumin in urine (68). The albuminuria may reflect impaired reabsorption of protein, as it occurs in association with cellular and tubular necrosis (115); however, increased filtration of albumin also may contribute to the albuminuria. If the endocytosis of filtered Hg–albumin is a significant pathway for entry of mercury into epithelial cells of the proximal tubule, then glomerular proteinuria induced by mercury may further exacerbate toxicity by increasing the delivery of Hg–albumin to the lumen of the proximal tubule.

Complexes between Hg$^{2+}$ and filtered proteins other than albumin also may be taken up by the proximal tubule epithelium. Rats administered Hg–metallothionein complex (Hg–Mt) accumulate more mercury in the renal cortex and outer medulla and excrete more mercury in urine than rats administered mercuric chloride, suggesting that the complex is more available for uptake in the proximal tubule than Hg$^{2+}$ bound to other endogenous ligands (113). Metallothionein is a 6,000–7,000-dalton, sulfhydryl-rich protein that forms highly stable complexes with Hg$^{2+}$, Cd$^{2+}$, and other divalent, thiolphilic metals (53, 54). The mechanism for the effect of metallothionein on mercury uptake has not been determined. Cd–metallothionein complex (Cd–Mt) is filtered at the glomerulus and undergoes reabsorption (32, 37), presumably in the proximal tubule where other low-molecular-weight proteins are known to be reabsorbed (67, 80). A similar mechanism may explain the increased uptake of mercury when Hg–metallothionein is administered to rats. Isolated perfused rabbit proximal tubule segments do not take up mercury when presented with Hg–Mt complex in the tubule lumen or on the basolateral side of the tubule, which is unfortunate because this preparation would be otherwise ideal for examining mechanisms of uptake of Hg–Mt (114). Possibly, the kinetics of endocytosis are too slow to be accommodated in this preparation, or the preparation lacks some necessary property for endocytosis of low-molecular-weight proteins. It also is possible that mercury impairs or inhibits endocytosis. In the rat, mercuric chloride has been shown to inhibit lysosomal catabolism of the low-molecular-weight protein, lysozyme (69). Examination of the transport and metabolism of other low-molecular-weight proteins (e.g., lysozyme, α1-microglobulin, retinol binding protein) in the isolated perfused tubule preparation would provide greater insight into these problems.

The extent to which metallothionein plays a role in the mercury nephropathy remains unresolved; however, its role in the pathogenesis of cadmium nephropathy has been much more thoroughly explored. Cd–Mt is highly toxic to the proximal tubule. Rats fed 1 mm cadmium chloride in drinking water ad libitum develop nephropathy after a 40–50-wk exposure (55) when the amount of cadmium in renal tissue exceeds 2 μmol Cd/g wet weight (~10 μmol Cd/g dry weight); however, a single injection of 2–10 μmol Cd/kg BW, as Cd–Mt (21, 95), produces nephropathy in rats within 4–12 hr when the amount of cadmium in renal tissue is only 0.1–0.5 μmol Cd/g wet weight (~0.5–2.5 μmol Cd/g dry weight). The mechanism by which Cd–Mt increases the toxicity of cadmium is not completely understood. Cd–Mt itself may be toxic, or the rapid extraction of Cd–Mt from the circulation by the kidney (89), endocytosis of Cd–Mt in the proximal tubule, and its subsequent degradation in lysosomes (16) may serve as a mechanism for delivering large amounts of Cd$^{2+}$ into proximal tubular cells where it can bind to and impair the function of critical proteins. Thus, it has been suggested that the development of cadmium nephropathy occurs when some critical amount of “free cadmium” (i.e., cadmium that is not bound to metallothionein) occurs in proximal tubule cells (78). Hg–Mt has not been shown to be more toxic than mercuric chloride; however, when mercury is administered as Hg–Mt, the segmental distribution of injury is shifted along the proximal tubule. The pars convoluta and early pars recta (S1 and S2) segments of the proximal tubule appear to be more vulnerable to Hg–Mt than is the late pars recta (S3) segment (18), whereas the opposite pattern of vulnerability is characteristic of mercuric chloride (73, 102, 119). It is tempting to speculate that this change in the location of the injury may reflect endocytosis of Hg–Mt in the pars convoluta segments of the proximal tubule. In support of this, the nephropathy induced by cadmium chloride or Cd–Mt is characteristically more severe in the pars convoluta than in the pars recta segments of the proximal tubule; however, an injected dose of cadmium chloride together with cysteine induces an acute injury predominantly in the pars recta segment (75). As previously noted, coadministration of cysteine with mercuric chloride increases the uptake of mercury in the renal cortex and OSOM where the pars recta segments of the proximal tubule are located (110).

Cellular Mechanisms of Mercury Toxicity

The disposition of mercury in cells is dominated by its high affinity for the thiolate anion and, therefore, potential molecular targets for mercury will include nonprotein and protein thiols. Hg$^{2+}$ binds to metallothionein and administration of mercuric chloride to rats increases the concentration of metallothionein in the renal cortex and OSOM (111). Pretreatment of rats with zinc, which induces the synthesis of metallothionein, affords protection against a subsequent acute nephrotoxic dose of mercuric chloride (112). Thus, metallothionein seems to serve as
a buffer for Hg\(^{2+}\) ions, presumably preventing its interaction with other critical sulfhydryls in the cell.

Hg\(^{2+}\) also complexes avidly with GSH. Treatment of rats with nontoxic to moderately nephrotoxic doses of mercuric chloride increases the GSH concentration in the renal cortex and OSOM (118). Renal proximal tubular epithelial cells, but not distal tubular cells, freshly isolated from rats treated with a nontoxic dose of mercuric chloride, show an increase in the activity of \(\gamma\)-glutamylcysteine synthetase, which catalyzes the rate-limiting step in the synthesis of GSH (62). Therefore, the increase in GSH induced by mercuric chloride appears to derive from an induction of the synthesis of GSH. In rats, depletion of GSH from the kidney by treatment with diethyl maleate (DEM) and buthionine sulfoximine (BSO), prior to a moderately toxic parenteral dose of mercuric chloride (4 mg/kg, \(-15\) \(\mu\)mol/kg), decreases the uptake of mercury in the renal cortex and increases the severity of nephrotoxicity (10). Thus, GSH, in addition to metallothionein, may serve as an important intracellular buffer for Hg\(^{2+}\).

The mechanism by which GSH depletion decreases mercury uptake in the kidney is not completely understood. The concentration of GSH in the kidney does not appear to be the sole determining factor. Zalups and Lash (121) pretreated rats with either DEM or BSO to produce similar levels of depletion of GSH (60% depletion) and similar GSH concentrations in the renal cortex or outer medulla and then administered a nontoxic dose of mercuric chloride (0.5 \(\mu\)mol/kg). Mercury uptake in the cortex and OSOM was significantly decreased by the DEM; however, BSO significantly decreased mercury uptake only in the OSOM and not in the renal cortex. Diethyl maleate depletes GSH in tissues by forming disulfide conjugates with GSH, whereas BSO depletes GSH by inhibiting the \(\gamma\)-glutamylcysteine synthetase step in the synthesis pathway for GSH. Therefore, it appears that depletion of GSH resulting from inhibition of the synthesis of GSH has less of an effect on mercury uptake in the renal cortex than does conjugation of GSH with DEM. By contrast, depletion of GSH by inhibiting GSH synthesis or conjugating GSH both decrease mercury uptake in the renal OSOM.

In contrast to the induction of GSH that occurs with nontoxic or moderately toxic doses, depletion of GSH from the kidney occurs in animals treated with severely toxic doses of mercuric chloride (1, 3, 39–42, 61, 118, 125). Depletion of GSH would not only reduce cellular Hg\(^{2+}\) buffering capacity but would expose the cell to oxidative damage, as GSH is an important free-radical scavenger. Mercury-induced depletion of renal GSH has been associated with increased free-radical-mediated oxidation of reduced porphyrins (100, 101). Depletion of cellular GSH in the kidney appears to occur in association with a general collapse of antioxidant mechanisms in the cell, including depletion of cellular ascorbic acid and vitamin E levels; and decreased activities of superoxide dismutase, catalase, GSH peroxidase, and GSSG reductase in renal cortex (22, 40, 42). All of these effects would imply severe oxidative stress in the renal cortex subsequent to a severely nephrotoxic dose of mercuric chloride.

The ATPases are a critical class of sulfhydryls in cells (84). In isolated, reconstituted preparations of renal medullary (Na\(^{+}\)–K\(^{+}\))-ATPase, Hg\(^{2+}\) binds to the ATPase, inhibits its activity and ATPase-driven transport (4, 5, 50). Binding of Hg\(^{2+}\) to the ATPase is reversible and occurs at a site distinct from the ouabain binding site (5) and appears to affect the interaction between the \(\alpha\) and \(\beta\) subunits of the ATPase protein complex (50). The binding site is on the cytosolic domain of the ATPase (4). The (Na\(^{+}\)–K\(^{+}\))-ATPase is the energy-requiring step in the development of the electrochemical gradients that drive solute and water transport in the proximal tubule. Inhibition of the (Na\(^{+}\)–K\(^{+}\))-ATPase would be expected not only to impair solute and water reabsorption in the proximal tubule but also impair the transport of substrates for energy metabolism and synthesis in the kidney (e.g., amino acids, citrate, fatty acids, glucose, lactate) (23). The above observations refer specifically to the plasma membrane (Na\(^{+}\)–K\(^{+}\))-ATPase; however, other ATPases, including the (Ca\(^{2+}\)–Mg\(^{2+}\))-ATPase and mitochondrial ATPases also may be targets of Hg\(^{2+}\), in which case, more diverse effects on cellular function might be anticipated.

Mercuric chloride depletes cellular ATP in the proximal tubule. In isolated microdissected segments of rat proximal tubule, incubation with 1 \(\mu\)M mercuric chloride depletes ATP only from the S2 segment and not from the S1 or S3 segments of the proximal tubule, thick ascending limb, or distal tubule (52). This pattern is consistent with greater \textit{in vivo} sensitivity of the \textit{pars recta} segment of the proximal tubule to injury. The mechanism for the ATP depletion derives, at least in part, from a direct impairment of mitochondrial respiration. \textit{In vitro} incubation with mercuric chloride decreases nystatin-stimulated oxygen consumption in suspensions of isolated rabbit proximal tubules. This effect occurs prior to the release of intracellular enzymes (i.e., lactate dehydrogenase), suggesting that the effect of mercury on mitochondrial function precedes and may contribute to irreversible cellular injury (117). Mercuric chloride has been shown to induce a variety of changes in function of mitochondria isolated from renal cortex. These include increases in state 4 oxygen consumption (i.e., uncoupled respiration) and decreases in state 3 (substrate-stimulated) oxygen consumption (97, 98); increases in calcium efflux and decreases in mitochondrial membrane potential (19, 20); and depletion of mitochondrial GSH, increased mitochondrial hydrogen peroxide production, and lipid peroxidation (65, 66). The above effects suggest that mercury, at sufficient doses, will induce a general collapse of oxidative metabolism and generation of ATP to support transport and synthesis.

A variety of critical cell functions depend on the regulation of the intracellular Ca\(^{2+}\) concentration. In primary cultures of renal tubular cells from rabbit cortex, incubation with mercuric chloride increases intracellular Ca\(^{2+}\) concentration (87). Exposures to 2.5–10 \(\mu\)M mercuric chloride resulted in a 2–10-fold increase in Ca\(^{2+}\) concentration, whereas, exposure to 25–100 \(\mu\)M mercuric chloride induced a biphasic change in Ca\(^{2+}\) concentration. The initial phase consists of a rapid rise in Ca\(^{2+}\) that is in-
dependent of extracellular Ca\(^{2+}\) concentration and presumably reflects release of Ca\(^{2+}\) from intracellular stores. This is followed by a decrease and subsequent gradual increase in intracellular Ca\(^{2+}\) that is dependent on extracellular Ca\(^{2+}\), which may indicate a change in permeability of the plasma membrane to Ca\(^{2+}\). These results indicate that mercuric chloride can perturb intracellular Ca\(^{2+}\) concentrations by at least 2 different mechanisms.

**Summary and Conclusions**

The picture that is emerging from these studies is that injury to the pars recta segment of the renal proximal tubule results from a direct effect of mercury accumulation in this region of the nephron. The mechanisms by which mercury enters cells of the renal proximal tubule remain obscure; however, it appears that pathways from both the luminal and basolateral side of the tubule are active. One pathway appears to be dependent on activity of the enzyme γGT; a second pathway appears to involve an organic anion transport mechanism. A common transport substrate for both mechanisms may be a Hg–cysteine complex. The exact mechanisms underlying the cellular degeneration associated with the accumulation of mercury in the pars recta segment of the proximal tubule have not been elucidated. Early biochemical changes induced by mercury include an induction of GSH and metallothionein that may be a direct effect of mercury on their metabolism or may represent a more general response to oxidative stress induced by mercury, possibly through impairment of membrane ATPase and/or mitochondrial function. At some point, or level of mercury in the cell, a multifocal collapse of cell metabolism occurs, including oxidative metabolism, cellular free-radical defense mechanisms, and membrane transport and permeability, initiating the terminal phases of cellular toxicity leading to cell degeneration and death. It is at this point that the effects of mercury on the tubular epithelium become evident histologically and the commonly applied urinary biomarkers reveal injury and functional impairment of the kidney.

While continued progress is being made in understanding the mechanism of toxicity of mercury and other metals, our current understanding remains frustratingly unsatisfactory. Mercury is illustrative in that we probably know more about the mechanisms of nephrotoxicity of inorganic mercuric mercury than we do of any other metal; yet many challenging problems remain. The exact mechanisms by which Hg\(^{2+}\), or its complexes, cross renal tubular cell membranes remain one of the more important unresolved problems. The same applies to all of the nephrotoxic heavy metals. The induction of GSH and metallothionein by mercury is intriguing; however, its precise role in the development of toxicity and the mechanism for the induction itself remain to be elucidated. The rise in intracellular Ca\(^{2+}\) that accompanies low-level exposures of renal tubular cells to mercury also is intriguing in view of the extreme importance of Ca\(^{2+}\) in signal transduction in the control of cellular metabolism. A mechanistic explanation for the effect of mercury on intracellular Ca\(^{2+}\) is not at hand. The mechanisms by which mercury disrupts mitochondrial functions needs to be elucidated if we are to understand the pathways that lead to catastrophic collapse of oxidative metabolism that occurs in mercury nephrotoxicity. These intriguing problems, for the most part, can be generalized to most of the nephrotoxic heavy metals. Further research applying the latest techniques for studying membrane transport and cellular metabolism should greatly improve our understanding of the mechanisms of nephrotoxicity of metals and of the pathophysiology of renal injury.

**References**


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